VARIOUS STORAGE TECHNIQUES OF PLUMS AND AVOCADOS

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Thesis presented in partial fulfilment of the requirements for the degree of
Master of Science in Agriculture
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University of Stellenbosch
South Africa

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December 2002

DECLARATION

I the undersigned hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or in part, been submitted at any university for a degree.

Signature Date

SUMMARY

Historically the storage of South African plum fruit involved cold storage at single low temperatures (-0.5°C). The ever present problem of internal breakdown, however, led to the development of the dual temperature storage regime (a type of intermittent warming) which was found to restrict the level of internal breakdown. However, this temperature regime led to the development of or unmasking of gel breakdown (GB).

During season one it was proposed that 'Songold' and 'Angeleno' plums could be stored at a single high temperature (7.5°C) during the shipping period (18 days) in combination with controlled atmospheres (CA). This was compared to the commercially used dual temperature regime (10 days at -0.5°C and eight days at 7.5°C). During season two the temperature regimes were adjusted to mirror the storage of the fruit from the date of harvest until the fruit is purchased. During the shipping period the dual temperature regime and the single high temperature regime were still compared. It was hypothesised that the fruit could be stored at the higher temperature in combination with CA during shipping, and this would allow the ripening of the fruit to be retarded, retaining good quality without exposure to the low, chilling inducing temperatures associated with the dual temperature regime.

The storage of both plum cultivars at the single high temperature in combination with CA had a positive influence on delaying the ripening of the fruit. Most noticeable was the delay of fruit softening and the restriction of colour and GB development of the 'Songold' plums. Most importantly, these results were achieved without the fruit being exposed to the low, injurious temperatures associated with the dual temperature regime. It was, however, concluded that due to the suppressed climacteric trait of these plums more benefit could be gained from the higher temperature storage in combination with CA on climacteric plums which display stronger ripening patterns.

In contrast to the plum industry, the use of CA in the storage of avocado fruit has risen to about 95% of the exported fruit. CA storage of avocados has shown positive results in retaining fruit firmness, restricting disorder development and extending the shelf life. Recently, research with 1-methylcyclopropene (1-MCP) storage of

avocados has been found to be as successful as CA storage and there is a belief that it will be the answer to storage for the avocado industry once registered for commercial use.

During the first experiment, 'Fuerte' and 'Hass' avocados were treated with CO_2 shocks (CO_2 levels which greatly exceed the initial intercellular concentrations of CO_2 are known as CO_2 shock treatments). These shock treatments were done over a series of three time periods and four CO_2 concentrations for the 'Fuerte' avocados and two time periods and two concentrations for the 'Hass' avocados. Results were disappointing, as the fruit treated with CO_2 were not able to retain their quality.

For the second experiment, 'Fuerte' and 'Hass' avocados were treated with either CA or 1-MCP, or CA and 1-MCP in combination. Previous research had identified a positive synergistic effect when CA and 1-MCP were combined, due to the fact that the treatments act at different points in the ethylene production process.

Both the CA and 1-MCP treatments, whether alone or in combination, had positive results in retaining firmness and extending the shelf life of the fruit. The treatments were also able to retain the quality of the 'Fuerte' avocados in terms of internal and external physiological disorders. 'Hass' avocados, however, are known to be of excellent internal quality and none of the treatments had a greater percentage of sound fruit than the fruit which were stored in air. The extended ripening gained by treatment with 1-MCP outweighed any increased loss due to fruit quality and in terms of the ease of application makes it superior to CA storage. However, before large-scale commercial application of 1-MCP begins, much still needs to be learned about its use on avocados.

Relative humidity (RH) control, forms an integral part of the storage of many fruit and vegetables. However, the difficulty in controlling and measuring RH has resulted in it being largely ignored. Research has shown that storage of fruit and vegetables at RH levels close to 95% has been able to restrict ripening and chilling injury development by decreasing the water stress on the fresh commodities during storage.

During the final experiment, 'Fuerte' and 'Hass' avocados were placed at the commercial storage temperature and a chilling temperature. At each temperature, the fruit were either stored under a high or a low RH. Results were inconclusive, as the higher RH showed no signs of restricting chilling injury or any other disorders in the fruit.

VERSKEIE OPBERGINGSTEGNIEKE VAN PRUIME EN AVOKADOS

OPSOMMING

In die verlede is pruime in Suid Afrika opgeberg volgens 'n enkeltemperatuur-regime (-0.5°C). Interne verval (IV) van die pruime was 'n probleem en het gelei tot die ontwikkeling van die dubbeltemperatuur-opberging regime wat IV verhoed het. Dit het egter gelei tot die ontwikkeling of ontmaskering van jelverval (JV).

Vir seisoen een is voorgestel dat die opberging van 'Songold' en 'Angeleno' pruime gedurende die verskepingstyd (18 dae), teen 'n hoë enkeltemperatuur van 7.5°C gekombineerd met beheerde atmosfeer (BA) geskied. Dit is vergelyk met die kommersiële dubbeltemperatuur-opberging regime (10 dae by -0.5°C en agt dae by 7.5°C). Vir seisoen twee is die temperatuur regime aangepas om die opberging van die vrug vanaf die oesdatum totdat dit deur die verbruiker gekoop word, na te boots. Tydens die verskepingstyd is die dubbeltemperatuur-opberging regime steeds vergelyk met die enkeltemperatuur opberging. Die gestelde hipotese is dat die vrugte gedurende verskeping by hoë enkeltemperatuur gekombineerd met BA opgeberg kon word. Sodoende word rypwording vertraag en die kwaliteit van die vrug behou sonder dat die vrugte blootgestel word aan temperature wat koueskade sal veroorsaak.

By beide kultivars het 'n hoë enkeltemperatuur, gekombineer met BA opberging, die rypwording van die vrugte vertraag. Mees opmerklik was die vertraging van die sagwording van altwee kultivars asook die vertraging van kleur ontwikkeling en JV van die 'Songold' pruime. Wat van belang is, is dat die resultate bereik is sonder dat die vrugte blootgestel was aan die lae temperature wat koueskade veroorsaak. Die onderdrukte klimakterium wat met 'Songold' en 'Angeleno' pruime verkry is toon dat pruim kultivars met sterker rypwordingspatrone meer sal baat deur opberging by hoë temperature saam met BA.

In teenstelling met die pruim bedryf, word omtrent 95% van die avokado vrugte wat deur Suid Afrika uitgevoer word onder BA verskeep. BA opberging toon vir baie jare al dat dit 'n positiewe invloed op die vertraging van sagwording, die behoud van gehalte en die verlenging van die raklewe van avokados het. Onlangse navorsing het

aangetoon dat 1-metielsiklopropeen (1-MCP) 'n produk is wat BA opberging se plek kan inneem. Daar word geglo dit is die antwoord vir avokado opberging in Suid Afrika.

Vir die eerste eksperiment het 'Fuerte' en 'Hass' avokados 'n CO_2 skok behandeling ontvang (CO_2 vlakke wat die interne sellulêre konsentrasie van CO_2 oorskrei). Die skok behandelings was oor drie tydperke en vier konsentrasie vlakke gedoen vir die 'Fuerte' avokados en oor twee tydperke en twee konsentrasies vir die 'Hass' avokados. Die resultate was teleurstellend omdat die vrugte wat met die CO_2 behandel is nie hul gehalte kon behou nie.

Gedurende die tweede eksperiment, was 'Fuerte' en 'Hass' avokados met BA of 1-MCP, alleen of in kombinasie behandel. Navorsing het bewys dat daar 'n dubbelle effek is as BA en 1-MCP saam gebruik word, omdat hulle etileen produksie deur verskillende maniere beheer word.

Beide die BA en 1-MCP behandelings, alleen of in kombinasie, het 'n positiewe effek uitgeoefen op die vrug deurdat dit fermheid behou en die raklewe verleng het. Die behandelinge het ook die kwaliteit van die 'Fuerte' avokados behou. 'Hass' is 'n avokado wat alreeds 'n baie goeie interne kwaliteit het. Vir dié rede het die vrugte wat net in lug opgeberg was die beste interne kwaliteit gehad. Maar die verlengde raklewe agv. die 1-MCP behandeling is van groter belang as die kwaliteit wat verloor is. Dit, saam met die feit dat die toepassing van 1-MCP behandeling baie makliker is as BA, maak dit die beter opsie. Voordat 1-MCP kommersiël geregistreer is moet daar egter nog baie geleer word oor die produk sodat dit so effektief as moontlik gebruik kan word vir die opberging van avokados.

Die beheer van relatiewe humiditeit (RH) word beskou as 'n groot veranderlike in die opberging van vrugte en groente. RH word egter meestal geïgnoreer in opberging omdat die beheer en meet daarvan moeilik is. Navorsing het al getoon dat indien die RH vlakke, gedurende opberging, naby aan 95% gehou word, kan koueskade verlaag word deur die water dampdruk tekorte op die vars produkte te verlaag.

Vir die finale eksperiment was 'Fuerte' en 'Hass' avokados opgeberg teen die kommersiële temperatuur sowel as 'n laer temperatuur. By beide temperature was die RH gewysig om laag of hoog te wees. Resultate was egter teleurstellend omdat die hoër RH nie koue skade of ander fisiologiese skade vertraag het nie.

DEDICATED TO MUM, PA, BARRY, CHRISTINE AND MATTHEW, FOR ALL THEIR LOVE AND SUPPORT DURING THE LAST SIX YEARS AND WITHOUT THEM THIS WOULD NOT HAVE BEEN POSSIBLE.

ACKNOWLEDGEMENTS

To the Lord my God, thank you for granting me the ability to achieve what I set out to achieve and more.

I wish to thank the following people and institutions for their contributions to the completion of this study:

To all my family for all their help, love and support.

To all the good friends I have made over the last six years, for all the support and great times, especially to Michelle and all the boys at the Pad.

Dr. M. Huysamer, Department of Horticultural Sciences, my supervisor for advice and guidance given and the assistance in preparing this thesis.

Dr. D.M. Holcroft, Department of Horticultural Sciences, Michigan State University, USA, my co-supervisor for all the patience, the help with planning and guidance.

Dr. M.C. Dodd, Alacer Aer Technologies, for help with planning.

Prof. K.I. Theron, Department of Horticultural Sciences, for input regarding statistical analysis of data.

Mr. A.B. Truter and Transfresh Africa, for the finance, guidance, knowledge, help and support which was invaluable and for the use of their facility for experiments.

Staff at the Department of Horticultural Sciences, for help with administration and for their friendship.

The Terrace, Pub and Grill, and to all the friends I made there for pulling me through all the tough times over the last six years.

TABLE OF CONTENT

1. INTRODUCTION

When considering the fresh produce which is exported from South Africa there is one commonality between all the products and that is a demand for a high quality commodity. As simple as it may sound it is still the one of the major factors restricting marketing of fresh produce on the overseas markets. The early use of controlled atmosphere (CA) storage in South Africa, around 1935 (Truter et al., 1982), was to accommodate off-season marketing, for the local market, by extending storage periods. However, more recently the focus of CA storage has moved to the improvement of quality and extension of the shelf life of fresh produce destined for the export market.

In 1993 Unifruco, the deciduous fruit export marketing organisation predicted that by the year 2000 the volume in plum production would increase to 4.9 million cartons (Taylor, 1993). Currently approximately five million cartons are exported of which only a fraction is exported under controlled atmosphere conditions. The main reason for this is the general belief that plum cultivars can be adequately exported with purely cold storage temperatures following recommended temperature regimes to counteract the development of internal browning and gel breakdown. This is true when considering the suppressed climacteric nature of some of the cultivars. Other cultivars are, however, more climacteric in nature and display stronger ripening patterns which may require more than just cold storage to retain the fruit quality for the export market. In this study we focus on storing late season plum cultivars under temperature regimes with longer time periods at higher temperatures in combination with CA. This is expected to deliver fruit of similar appearance and firmness as the commercial storage method while delivering improved internal quality due to the shorter time spent at the lower temperatures which have proven to be injurious.

The South African avocado industry has made huge progress since the first commercial orchard was planted by Dr. Merensky in the 1930's (Toerien et al., 1992). This progress led to the establishment of the South African Avocado Growers Association (SAAGA) in 1967 (Toerien et al., 1992). During the early 1990's the introduction of a new cultivar, 'Pinkerton' boosted industry expectations to the extent that it was predicted that by the year 2000, 20 million cartons would be exported from

South Africa (Toerien, 1994). Although this may have been true considering the numbers of trees of the new cultivar which were being planted and the yields it was delivering. 'Pinkerton' could, however, not endure the cold storage period required for export. Thus SAAGA predicts for the year 2002 that approximately 9.5 million cartons will be exported. The avocado industry relies heavily on CA as a storage method during exporting of avocados. This is seen by the fact that approximately 95% of exported fruit are shipped under CA conditions.

The primary reason for the use of CA storage for avocados is due to the fruits susceptibility to chilling injury. Thus the fruit cannot be stored at low chilling temperatures due to damage which would occur and at higher temperatures the fruit will ripen too quickly and decay could set in before reaching the export market. Thus CA storage allows the fruit to be stored at between 5 - 13°C (Kader, 1997) whilst keeping the quality and extending the shelf life of the fruit.

An ethylene antagonist, 1-methylcyclopropene (1-MCP), has been identified as a potential replacement for CA storage of avocados. This product is a competitive inhibitor of ethylene action (De Wild et al., 1999) and acts by binding to the ethylene receptor with more affinity than ethylene itself (Rupasinghe et al., 2000). Furthermore, once registered for commercial use, it is expected to be far more cost effective than CA storage and easier to apply. The aim of this study is to identify which method is more effective for storage of export avocados, either CA or 1-MCP, in terms of firmness retention, fruit quality and shelf life extension.

When considering storage methods such as CA and 1-MCP it is often forgotten that the effectiveness of the storage is also very reliant on other factors such as relative humidity (RH). The reason for this is that over the years the control and measurement of RH has been very difficult (Gaffney, 1978). Despite this, much research has shown that controlling RH to the ideal level for a specific commodity can effectively retain firmness (Ben-Yehoshua et al., 1979), restrict colour (Littmann, 1972) and disorder development (Chien et al., 1998) and extend shelf life (Littmann, 1972) by reducing the water stress placed on the commodity. This study aims to identify the effectiveness of higher RH on retaining fruit quality at different temperatures during the storage of avocados.

In summary, the experiments conducted for the purpose of this thesis were aimed at identifying the most effective method of storage for the export commodities, plums and avocados.

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2. LITERATURE REVIEW: Various storage techniques of stone fruit, avocados and other fruit and vegetables

2.1 Introduction

2.1.1 Controlled atmosphere storage

The technique of modifying atmospheric composition for use in agriculture likely dates back thousands of years previously in the Middle East (Kays as reported by Beaudry, 1999). The first controlled atmosphere cold stores in South Africa were built by the Montelo Brothers, in 1935, and Elgin Fruit Packers, in 1936 (Truter et al., 1982). During the late seventies apples were cold stored under regular atmosphere conditions for up to six months after which deterioration set in (Truter et al., 1994a). Subsequent to that controlled atmosphere storage has become an important method of storage in the South African fruit industry to accommodate off-season marketing by storage for longer periods (Truter et al., 1994a).

Previous to 1980 ultra-low oxygen levels had shown to extend the storage life of 'Cox's Orange Pippins' apples by up to two months but according to Lovelidge (1981) the overriding problem at that stage was maintenance of the low oxygen levels (1.1 - 1.4%). That problem was solved with the development of an automatic oxygen control system (Lovelidge, 1981). Subsequent to that restriction much progress has been made in controlled and modified atmosphere technology of fruit and vegetables.

Controlled atmosphere (CA) and modified atmosphere (MA) storage technology provide a means of slowing ripening and senescence of many fresh fruits and vegetables during storage, transport and marketing (Mattheis and Fellman, 2000). This is not only done by refrigeration but also by changing the atmospheric composition within the storage environment. CA storage involves the controlled setting and maintenance of an atmospheric environment by filling a container with the desired mixture of oxygen, carbon dioxide and nitrogen which forms the balance of the atmosphere. Maintenance of the atmosphere is achieved by ventilation with air or by CO_2 scrubbing. In contrast, MA relies on the respiration of the commodity to modify the atmosphere in which it is stored. In other words respiration will use the

O_2 and add to the CO_2 within the storage environment. This is usually done by modified atmosphere packaging (MAP) and is currently applied with the use of polymeric films (Kader and Watkins, 2000). MAP relies on the permeability of the film, respiration, temperature and other factors to maintain the atmosphere composition (Smock, 1979). MA and CA influence many aspects of produce quality from colour changes, nutritive value, flavour, aroma, textural changes and softening (Mattheis and Fellman, 2000). Gas mixtures which will provide the optimum storage conditions will depend on factors such as crop and variety but usually O_2 levels are decreased to 2 to 3% and CO_2 increased to 5 to 15% (Idler, 1997). It was shown that attainment of CA conditions is less critical when cooling is rapid and effective (Van Eeden et al., 1988). CA conditions for effective storage of 'Bon Chretien' and 'Packham's Triumph' pears must be attained within nine days (Van Eeden et al., 1988).

As far as primary metabolism (ie. processes of glycolysis and fermentation) is concerned, all processes are affected by both lowered O_2 and increased CO_2 levels (Mathooko, 1996b; Olsen, 1982). This is seen by the well documented fact that respiration rates (ie. O_2 uptake) are slowed with these changes in atmospheric composition (Beaudry, 1999; Beaudry, 2000; Kader, 1986).

The activities of the two main enzymes involved in ethylene production (secondary metabolism process), viz. 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase, are both affected by increased CO_2 levels whether in combination with lowered O_2 levels or not (Mathooko, 1996a). It was originally thought that increased CO_2 levels acted in the same way as 1-methylcyclopropene (1-MCP), as a competitive inhibitor of ethylene action (Burg and Burg, 1967). De Wild et al. (1999), however, proved that CO_2 acted more as a non-competitive inhibitor affecting either ACC synthase or ACC oxidase in the preliminary stages of ethylene production resulting in a synergistic effect when the two treatments were combined.

The O_2 lower limit and CO_2 upper limit which commodities can tolerate has been a topic of debate for many years. Once these limits for O_2 (Beaudry and Gran, 1993) and CO_2 (Thomas, as reported by Beaudry and Gran, 1993) are exceeded for a particular organ fermentation and off-flavours can occur. Thus CO_2 injury is a factor

which must be taken into consideration. Susceptibility to this injury is affected by the following:

- The first several weeks of CA storage of apples is vital (Watkins, 2000). Therefore strict control (Watkins, 1997) and timing of exposure is critical.
- If apples are exposed to air storage for a few days prior to elevated CO_2 storage, susceptibility to injury can be restricted (Colgan et al., 1999; Elgar et al., 1998).
- Treatment with DPA (diphenylamine), in a postharvest drench, can prevent CO_2 injury in apples (Watkins et al., 1997).
- 'Bartlett' pears became less tolerable to increased CO_2 levels with increases in storage temperature (Ke et al., 1990).

It must be born in mind that the various processes of ripening and senescence do not have the same O_2 and CO_2 optima for maximum beneficial responses (Mattheis and Fellman, 2000). Subjecting fruit or vegetables to atmospheres outside the optimum levels of O_2 and CO_2, for a specific commodity, could result in irregular ripening, initiation or aggravation of certain physiological disorders, increased susceptibility to decay as well as development of off-flavours (Watkins, 2000). To address this problem the employment of the one-size-fits-all process must be discarded, for a process which can differentiate between variations in product responses as related to cultivar or postharvest effects (Watkins, 2000). Thus it is important to quantify plant responses to atmosphere modification as new technologies are being developed (Beaudry, 1999). Furthermore it was suggested by Dadzie et al. (1996) that when considering modified atmosphere storage fruit responses to CA or MAP are likely to be more consistent when defined in terms of internal atmosphere composition of the fruit rather than external atmosphere composition to which the fruit are exposed. This is because the internal atmosphere composition is always different from the composition of the external atmosphere (Dadzie et al., 1993) and is thus less directly linked to the substrate availability for both respiration and ethylene production (Dadzie et al., 1996).

The use of CA storage for a quarantine procedure for postharvest insect control has been extensively investigated (Ke and Kader, 1992). This involves exposure of the

fruit to low O_2 (less than 1%) and high CO_2 (greater than 50%) (Ke and Kader, 1992). The fruit concerned must, however, be able to tolerate the extreme atmospheric conditions for longer than it would be expected to kill the insects concerned (Ke and Kader, 1992).

To date CA containerisation for ocean transport has not been utilised within industry to a large extent. Dohring (1997) reported that in 1996 only 2.5% of the 164 000 twelve metre containers which arrived in the US were transported under CA. Furthermore, that 2.5% represented over 75% of the entire containerised CA market. This is primarily because there are only a handful of commodities which can reap the benefit of the extra cost involved with CA storage (Dohring, 1997).

2.1.2 1-methylcyclopropene storage

Much research has gone into the prevention of ethylene action in plants. Recently this has been achieved with an odourless, gaseous inhibitor, 1-methylcyclopropene (1-MCP). This product which antagonises ethylene action has allowed the ripening processes which are dependent on ethylene to be identified and differentiated (Golding et al., 1998). The molecular formula of 1-MCP is: C_4H_6.

It was originally thought that 1-MCP worked by inhibiting the increased activity of the ethylene biosynthetic enzymes, ACC synthase and ACC oxidase during ripening (Nakatsuka, as reported by Golding et al., 1998). It was, however, proven to be a competitive inhibitor of ethylene at the active binding site thus blocking ethylene action (De Wild et al., 1999; Rupasinghe et al., 2000a; White et al., 2001). Furthermore, 1-MCP blocks the normal feedback regulation of ethylene, therefore delaying ripening of the fruit (Dong et al., 2001b; Golding et al., 1998). The application of 1-MCP must be done before autocatalytic ethylene production is measurable for it to be most effective otherwise it will be too late to affect the progress of the climacteric (Golding et al., 1998).

Plants can eventually overcome the inhibition by 1-MCP by making new ethylene receptors (Sisler et al., 1996). 1-MCP has more affinity to the ethylene receptor than ethylene itself, thus 1-MCP treatments are effective in very low dosages

(in the $nl.l^{-1}$ range) (Rupasinghe et al., 2000a). It has also been found that treatment with 1-MCP at lower temperatures requires higher concentrations of 1-MCP for the same result (Nazir et al., 2001; Sisler et al., 1996). Thus, for this reason, treatments with 1-MCP are generally done at room temperature.

The reaction time of 1-MCP is varied. Carnations treated with 1-MCP remained insensitive for 12 - 15 days at 24°C (Sisler and Serek, 1997) while banana fruit treated with 1-MCP remained insensitive for 10 - 11 days after the control fruit stored in air displayed increases in respiration and ethylene production (Golding et al., 1998). Ripening of apples can be inhibited for as long as 25 - 35 days by a single exposure to 1-MCP compared to control fruit at room temperature (Fan et al., 1999).

While Fan et al. (1999) discussed the effectiveness of a single exposure to 1-MCP at room temperature it was found by Nazir et al. (2001) that at elevated temperatures (≥ 5°C) 1-MCP effectiveness can be improved by frequent applications. Furthermore this study suggested that the reliance on refrigeration for apple storage could be reduced by frequent 1-MCP application at elevated temperatures.

2.2 Controlled atmosphere and 1-MCP storage of stone fruit, avocado and other fruit and vegetables

2.2.1 History of plum and avocado storage in South Africa

2.2.1.1 Plum

During the 1930's complaints about South African plums revolved around variability in maturity and use in cooking was the only hope for further trade to the United Kingdom (Davies et al., as reported by Taylor, 1996). This resulted in the replacement of the commonly used single temperature storage at -0.5°C with a dual temperature storage regime as research had shown that quality was better (Boyes and De Villiers, as reported by Taylor, 1996). This is a type of intermittent warming and was able to reduce internal breakdown (IB), a chilling injury (CI) disorder, for some cultivars (Boyes and De Villiers, as reported by Taylor, 1996). The problem with the dual temperature regime was that when shipping to North America fruit had to be held

at -0.5°C to sterilise against fruit fly (Boyes et al., 1952), which meant fruit being more prone to IB. Thus, for effective use of the dual temperature storage regime it was determined during the 1950's that certain cultivars could not be stored at -0.5°C for longer than 10 days (Ginsburg, as reported by Taylor, 1996).

IB becomes apparent when internal tissues become translucent (Eksteen et al., 1986). Further progress results in darkening of the tissues until dark-brown to black (Eksteen et al., 1986). Since 'Songold' plums were found to be susceptible to IB (Eksteen, 1982), the dual temperature regime was adopted as it was found that the disorder could be prevented (Hartmann et al., 1988). Kotzé et al. (1989) proved that an increase in temperature at some stage during the storage period can decrease the incidence of IB. However, the dual temperature regime was not able to solve the problem of gel breakdown (GB), which was possibly unmasked with the application of the dual temperatures. GB is the gelatinous breakdown of the mesocarp tissue between the stone and the middle of the mesocarp (Taylor et al., 1993a). These disorders are only visible internally thus an attractive fruit may still be of very poor quality and most importantly of low economic value (Eksteen et al., 1986; Kotzé et al., 1989).

Currently for export of plum fruit to the overseas market a realistic time frame would involve up to five weeks storage for early cultivars and six weeks for late cultivars, from the date of harvest. Fruit would generally be stored for ± 10 days at -0.5°C from the time of harvest. This would constitute about eight days for the fruit to be delivered at the harbour and about two days at sea. Thereafter temperatures would be increased to 7.5°C for a time period depending on the cultivar and lower again to -0.5°C for the remainder of the shipping. Therefore cultivars which display strong ripening capabilities would be stored under a PD 4 (four days at 7.5°C followed by the remainder of the voyage at -0.5°C) temperature regime and cultivars with restricted ripening abilities under a PD 8 (eight days at 7.5°C followed by the remainder of the voyage at -0.5°C) temperature regime. From the arrival time at the overseas harbour till arrival at the market early plum cultivars would be stored for about two weeks and late cultivars for about three weeks at -0.5°C. Thereafter shelf life temperatures vary from 15 to 20°C.

Truter et al. (1994b) found that 'Songold' plum fruit stored for seven weeks under intermittent warming performed best under CA conditions of 3% O_2 and 5% CO_2 for the full storage time. This was opposed to storage under partial CA of 2% O_2 and 5% CO_2 for the first 10 days of storage after which the fruit were held under regular atmosphere conditions. Only 4.5% GB occurred after seven weeks of CA storage, while control fruit showed decay after five weeks. 'Casselman' plum fruit stored under CA reacted similarly to the 'Songold' plums, having 3.4% GB and mean firmness of 5.1 kg after eight weeks storage. Truter et al. (1994b) also investigated high temperature conditioning (storage at 20°C under a gas mixture of 21% O_2 and 5% CO_2 for two days and afterwards seven weeks of intermittent warming under regular atmosphere conditions), CA storage (3% O_2 and 5% CO_2) and partial CA storage of 'Laetitia' plums for eight weeks and a further week at 10°C shelf life temperature. After eight weeks storage the fruit had no IB.

In contrast Eksteen et al. (1986) found that O_2 levels between 2 - 6% and CO_2 levels between 2 - 10% on 'Santa Rosa' and 'Songold' plums stored at -0.5°C had no effect on controlling IB, extending the cold storage period or maintaining fruit quality over a storage period of three to six weeks. The recommended atmospheric composition for storage of plums is 1 - 2% O_2 and 0 - 5% CO_2 (Kader, 1997).

2.2.1.2 Avocado

Avocado history in South Africa dates back to around 1904 with only a few seedling trees of West Indian origin (Toerien et al., 1992). By 1925 budded Mexican and Guatemalan avocado trees had been introduced to a few nurseries by the Department of Agriculture (Toerien et al., 1992). One of the first small commercial orchards planted was by Dr. Merensky in the 1930's but the industry began rapid development in the 1960's as a replacement crop for disease infested citrus (Toerien et al., 1992). This development led to the formation of the South African Avocado Growers' Association (SAAGA) in 1967 (Toerien et al., 1992).

It has become very common for South African grown avocados to arrive at the overseas market at the incorrect stage of maturity (ie. under ripe or overripe). This is partly due to the fact that very often fruit are in transit for more than 30 days, from the

date of harvest, before reaching the export market (Couey, 1982). In previous years the major method to prolong the shelf life of South African grown avocados was by low temperature storage (Bezuidenhout et al., 1992). According to Bezuidenhout et al. (1992) to gain success in cold storage knowledge and understanding of fruit physiology, cold storage and effective postharvest management is required. By applying a temperature management system, improved firmness and quality was achieved during the 1989 / 90 seasons (Bezuidenhout et al., 1992). This temperature management system involved declining temperatures during shipment ranging between 3.5°C to 7.5°C and could accommodate early season fruit which were found to be more sensitive to low temperatures (Bezuidenhout et al., 1992). This system could also be varied to accommodate fruit maturity (moisture content) and climacteric stage. Currently by monitoring the moisture content of the fruit late season fruit are still being stored at lower temperatures than early fruit. This can be described as a form of temperature conditioning to alleviate CI.

Thus the option of storing avocados at very low temperatures to restrict ripening has long since been discarded due to susceptibility of the fruit to CI (Couey, 1982). This has opened the door for storage at higher temperatures of between 5 - 13°C (Kader, 1997) in combination with CA or 1-MCP. According to Smock (1979) the first CA storage of avocados was done at the University of California, Los Angeles in the early 1940's. Kader (1997) recommends O_2 levels between 2 - 5% and CO_2 level between 3 - 10% for storage of avocados. Furthermore due to avocado fruits sensitivity to ethylene it has also been found that removal of ethylene from the storage environment, especially in combination with CA storage, has shown further promise in extending the shelf life of avocados (Hatton and Reeder, 1972).

Truter and Eksteen (1987) stated that the orderly marketing of South African avocados on the export market requires fruit to be ripe within four to five days after arrival and sold before the next consignment arrives. They found that a CA composition of 2% O_2 and 10% CO_2 was able to extend the shelf life of 'Fuerte' avocados to 10 days but there was a concomitant increase in anthracnose rot. It was for that reason that ripening of four days was preferred so as to prevent the onset of decay (Truter and Eksteen, 1987). Truter and Eksteen (1987) also found that the CA

was able to restrict CI, pulp spot and grey pulp when the fruit were stored at 5.5°C but delayed ripening to much too be of any value.

Currently approximately 95% of the exported avocados from South Africa are stored and transported under CA conditions. The primary reason for the use of CA, as with the temperature management system, is to improve firmness, shelf life and to restrict CI and decay disorders of the fruit. The identification of 1-MCP as a potential inhibitor of avocado fruit ripening has become a recent reality. It has proven to work as effectively as CA storage at much lower cost. It is set to be registered for use in the near future in South Africa and may be the answer to long term storage of avocados. However, much still needs to be learned before then to make sure it is used as effectively as possible.

2.2.2 Colour development

Controlled atmosphere storage: General

Chlorophyll degradation is accelerated by ethylene, inducing yellowing of green tissues and thus reducing market quality (Abeles as reported by Nazir et al., 2001; Kader, 1985). It was found by Poenicke et al. (1977) that cucumber chlorophyll content was decreased on exposure to 0.1 - 10 $\mu l.l^{-1}$ ethylene treatments.

Controlled atmosphere conditions have a large impact on colour changes during ripening and senescence, particularly the changes from green to yellow (Beaudry, 2000; Kader, 1986; Mattheis and Fellman, 2000). Thus the high CO_2 and low O_2 conditions reduce losses of chlorophyll, thus restricting quality loss (Barth et al., 1993; Bhowmik and Pan, 1992). Storage of 'Golden Delicious' apples (Eksteen and Truter, 1986; Truter et al., 1982), 'Granny Smith' apples (Truter et al., 1982), 'Starking' apples (Truter et al., 1982) 'Packham's Triumph' pears (Eksteen and Truter, 1986; Truter, 1990) and 'Dwarf Cavendish' bananas (Bower, 1986) under CA conditions restricted the loss of green colour when compared to the control fruit.

In two separate experiments done on broccoli it was found that increased CO_2 levels (Makhlouf et al., 1989) and 1-MCP (Ku and Wills, 1999) restricted yellowing,

reiterating that elevated CO_2 levels and 1-MCP have a similar effect on ethylene synthesis. It was found by Salunkhe and Wu (1973) that chlorophyll loss of tomatoes was more delayed with lower O_2 until, at 1% O_2, loss of chlorophyll was completely inhibited for one month after which degradation started. This response results due to the inhibitory effect O_2 places on ethylene dependent processes (Matile et al. as reported by Beaudry, 2000).

Controlled atmosphere storage: Stone fruit

Retamales et al. (1992) found that high CO_2 concentrations during CA storage had more of an effect in delaying colour development of nectarines than low O_2 concentrations. Exposure of 'Wickson' plums to 30, 50 and 75 kPa O_2 and ripening at 20°C delayed colour changes associated with ripening (Claypool and Allen, as reported by Kader and Ben-Yehoshua, 2000). Darkening of the red pigment of 'Bing' cherries was retarded by storage under 0.25% or 0.02% O_2 at 5°C (Ke and Kader, 1992).

'Tyrintos' and 'Boccuccia Spinosa' apricots were treated with 100% CO_2 for 48 hours at 18°C and thereafter kept at 18°C in air for seven and four days, respectively (Garosi et al., 1997). These fruit maintained their colour better with smaller changes in lightness and larger changes in chroma than the control fruit which were stored for the duration of the experiment at 5°C. By exposure of 'Bühler Frühzwetsche' plums to 12% CO_2 and 2% O_2 the best retention of initial colour was achieved after four weeks storage compared to fruit stored in air (Streif, 1989).

1-MCP storage: General

Chlorophyll content of 'Redchief Delicious' apples was maintained by frequent applications of 1-MCP, further supporting that ethylene plays a major role in chlorophyll degradation (Nazir et al., 2001). Treatment of bananas with 1-MCP also influences fruit colouring (Golding et al., 1998).

2.2.3 Firmness

General

Softening of fruit is regarded as the ripening process which is the most sensitive to ethylene (Lelièvre et al., 1997) and this softening is bound to reduce storability (Kader, 1985). This was proven by Gerasopoulos and Richardson (1996) with experiments on pears where fruit treated with low levels of propylene (an ethylene analogue) softened but the other aspects of ripening were not induced. Watermelons treated with 5, 30 or 60 $\mu l.l^{-1}$ ethylene reduced firmness and accelerated deterioration when compared to fruit not treated with ethylene (Risse and Hatton, 1982).

Firmness of fruit is also influenced by the storage temperature. This is supported by Kader (2002) due to the relationship between temperature and respiration named Q_{10}, as is described in section 2.2.6. 'Sungrand' nectarines lost 17% of initial firmness during eight weeks storage at 2.2°C and 78% of initial firmness during the same storage time at 5°C (Mitchell, 1986). Mitchell (1986) concluded that this was partly due to reduced ethylene activity at these lowered temperatures.

The key enzymes which catalyse the hydrolysis of cell wall pectin are polygalacturonase (PG) and pectinesterase (PE) (Dong et al., 2001a). PG activity in 'Flavortop' nectarines was very low at harvest while PE activity was high and the reverse occurred as fruit ripened (Dong et al., 2001a). It has also been found that PG is ethylene regulated (Sitrit and Bennett, 1998).

Controlled atmosphere storage: General

Within the tolerance range of CO_2 and O_2 the softening of fruit can be restricted (Lougheed and Dewey, 1966) but outside of the tolerance range softening can be accelerated (Nanos and Mitchell, 1991). Knee found that apples stored under CA had flesh softening rates half of maximal at 2.5 - 4.0% O_2 (as reported by Kader, 1986). CA storage of 'Packhams Triumph' pears (Truter, 1990), and 'Golden Delicious', 'Starking' and 'Granny Smith' apples restricted the loss of firmness when compared to the control fruit (Eksteen and Truter, 1986; Truter et al., 1982).

The extreme levels of carbon dioxide in CO_2 shock treatments (CO_2 levels which greatly exceed the initial intercellular concentrations of CO_2 are known as CO_2 shock treatments) results in a build up in CO_2 within the fruit via diffusion. These increased levels of CO_2 prevent extensive loss of sugars and overripening and thus delay fruit softening (Salisbury and Ross, 1991a). In a storage experiment with kiwifruit, fruit were treated with two, four and six intermittent exposures to 30% CO_2 stored at 0°C and compared to an air treatment (Nicolas et al., 1989). In all cases a significant effect in delaying fruit softening was observed and the effect increased with the number of exposures to high CO_2.

At high levels of O_2 a slight inhibiting effect on firmness of 'Braeburn' apples by CO_2 became visible (Hertog et al., 2001). Furthermore it was found that lowering of O_2 levels resulted in increasingly firmer fruit. However, at extremely low O_2 levels there was a clear softening of the fruit. Hertog et al. (2001) suggested that the stage where oxidative respiration is inhibited and fermentation increases, could have an effect on loss of firmness. By using Michaelis Menten type gas exchange models Hertog et al. (2001) was able to support their hypothesis that softening processes of the fruit are closely linked metabolically to gas exchange. This was supported by Ke et al. (1990) who found that treatment of 'Bartlett' pears with 0.25% O_2 significantly reduced respiration rates but when O_2 levels were reduced to 0.03% there was an increase in respiration rate.

Truter (1987) discussed the effect of CA (3% O_2, 0% CO_2) on the quality of 'Bon Chretien' pears stored for eight, 12 and 16 weeks at -0.5°C followed by four days at 20°C. There was no significant difference in firmness after the three time periods but after 16 weeks storage all the fruit had IB.

Controlled atmosphere storage: Stone fruit

The storage of stone fruit such as nectarines (Levin et al., 1995; Lurie et al., 1993; Retamales et al., 1992; Rushing, 1993; Tain et al., 1996; Truter et al., 1994c), peaches (Rushing, 1993) and plums (Rushing, 1993; Streif, 1989; Truter and Combrink, 1997)

under CA conditions (10 - 12% CO_2 and 0.3 - 15% O_2) has proven to have a positive effect on retaining the firmness of the fruit.

Truter et al. (1994c) tested the effect of intermittent warming during CA storage and a high temperature conditioning treatment, which involved storage under high CO_2 levels for two days at 20°C followed by storage at -0.5°C, versus a control treatment on 'Olympia', 'Zaigina', 'Nectared 9' and 'Flamekist' nectarines. Storage occurred for four, five, six, seven and eight weeks for each treatment. The 'Olympia' and 'Zaigina' control treated fruit generally had the most firmness retention over the five storage times while the fruit treated with intermittent warming and CA softened fastest. In contrast, the 'Nectared 9' nectarines treated with CA under intermittent warming softened the least over the five storage times while at most of the storage times the control fruit had firmness levels very close to 0 kg. Whether these firmness differences were significant was not stated.

In a land-based container experiment in Chile seven peach cultivars, 13 nectarine cultivars and nine plum cultivars were cold stored with or without CA followed by four days in ambient atmosphere as shelf life treatment (Rushing, 1993). Firmness was the quality factor which was most influenced by the CA treatment. Five of the peach cultivars, 10 nectarine cultivars, and six plum cultivars treated with CA had significantly firmer fruit than the untreated fruit after the storage period. After the shelf life period six of the peach cultivars, eight nectarine cultivars, and five plum cultivars treated with CA had significantly firmer fruit than the untreated fruit.

General: Avocados

Awad and Young (1979) found that PG activity was not detected at the preclimacteric stage of 'Fuerte' avocados but during ripening (postclimacteric) cellulase and PG activity increased while PE activity decreased. Furthermore it was suggested that cellulase appeared to be responsible for the early stages of avocado fruit softening and PG for final fruit softening (Awad and Young, 1979; Bower and Cutting, 1988).

Controlled atmosphere storage: Avocados

CA storage has been found to have a positive influence on avocado fruit softening (Corrales-Garcia, 1997; Hatton and Reeder, 1972; Jordan and Smith, 1993; Lizana and Figueroa, 1997; Meir et al., 1995; Meir et al., 1998; Pesis et al., 1994; Pesis et al., 1994; Eksteen and Truter, 1985; Truter and Eksteen, 1987). Meir et al. (1998) found that the firmness of 'Fuerte' avocados could be retained for 10.5 weeks storage at 5°C with increased CO_2 concentration and decreased O_2 concentration.

At a concentration of 21% O_2, the higher the CO_2 concentration the longer the 'Hass' avocados remained firm during storage (Meir et al., 1995). When the O_2 concentration was decreased to 3% in combination with either 0.5, 3 or 8% CO_2, fruit softening was delayed (Meir et al., 1995). Meir et al. (1995) also concluded that the higher the CO_2 concentration, which they used, during the storage time the longer the fruit took to soften during the shelf life period.

'Hass' avocados stored in CO_2 enriched atmospheres (20% CO_2 and 17% O_2 or 40% CO_2 and 13% O_2) or in air softened similarly during five days storage at 20°C (Lange and Kader, 1997). However, five days at 20°C was not long enough for softening to occur in preclimacteric 'Hass' avocados.

1-MCP storage: General

The prevention of ethylene action by 1-MCP means that 1-MCP treated fruit would be firmer than untreated fruit as ethylene is directly involved in fruit softening (Rupasinghe et al., 2000a). This action of 1-MCP on firmness was found on apples (Rupasinghe et al., 2000a; Rupasinghe et al., 2000b), 'Red Rosa' plums (Dong et al., 2001b), 'Flavortop' nectarine (Dong et al., 2001a), 'Hass' avocados (White et al., 2001) and 'Quintal' avocados (Kluge et al., 2002). This was consistent with what Nazir et al. (2001) found on 'Redchief Delicious' apples but they suggested that the use of 1-MCP for storage of apples could be more cost effective by more frequent applications at elevated temperatures ($\geq$ 5°C) and so reduce the reliance on refrigeration.

2.2.4 Total soluble solids, titratable acidity, flavour and aroma

Controlled atmosphere storage: General

The sugar to acid ratio in combination with the aromatic compounds or volatiles determines the internal quality of fruit (Selli and Sansavini, 1995). The inhibition of starch degradation of tomato fruit by low O_2 (1 - 10%) atmospheric storage results in inhibited sugar formation (Salunkhe and Wu, 1973). Truter (1990) found that CA storage of 'Winter Nelis' pears and 'Forelle' pears lowered the TSS of the fruit.

If anaerobic conditions develop, due to extreme levels of O_2 and CO_2, losses of sugar and titratable acids can be enhanced and off-flavours may develop (Mattheis and Fellman, 2000). These off-flavours are a result of the accumulation of ethanol and acetaldehyde and altered production of other compounds which contribute to flavour and aroma (Beaudry, 2000; Mattheis and Fellman, 2000; Tian et al., 1996).

CA storage reduces losses in acidity in fresh fruits (Kader, 1986). According to Goodenough and Thomas (as reported by Kader, 1986), CA conditions slowed down the losses in sugars and organic acids in tomatoes during storage at 12.5°C for up to two months. Retention of acidity was also achieved in 'Golden Delicious' apples (Eksteen and Truter, 1986; Lau and Looney, 1982; Truter et al., 1982), 'Starking' apples (Eksteen and Truter, 1986; Truter et al., 1982), 'Granny Smith' apples (Eksteen and Truter, 1986; Truter et al., 1982), 'Bon Chretien' pears (Truter, 1987), 'Packhams Triumph' pears (Eksteen and Truter, 1986; Truter, 1990), 'Doyenne du Comice' pears (Truter, 1990), 'Winter Nelis' pears (Truter, 1990) and 'Forelle' pears (Truter, 1990) by storage under CA conditions when compared to the control fruit.

Volatile production which contributes to apple flavour and aroma can also be reduced by storage under CA. However, the degree of reduction as well as the extent of recovery are influenced by the duration of CA storage (Mattheis and Fellman, 2000).

Controlled atmosphere storage: Stone fruit

Levin et al. (1995) found there was no consistent effect on soluble solids content of 'Fiesta Red' nectarines by changes in the storage atmosphere. In contrast to this 'Bühler Frühzwetsche' plums retained their acidity and sugar content after four weeks under CA storage (12% CO_2 and 2% O_2) (Streif, 1989). Similarly 'Independence' nectarines stored under low oxygen (0.3%) at 0°C or 6°C retained acidity throughout storage compared to the control fruit (Tian et al., 1996). The exposure of cherries to O_2 levels as low as 0.02% caused accumulation of ethanol and thus a slight alcoholic off-flavour (Ke and Kader, 1992).

1-MCP storage: General

The effect of 1-MCP on soluble solid content of early, mid and late season apple cultivars stored in air or CA was inconsistent while the TA was always higher than those of the control fruit (Watkins et al., 2000). It was further suggested by Nazir et al. (2001) that since elevated temperatures allowed acidity loss despite 1-MCP application fruit may retain firmness but could develop an insipid taste after extended storage. Rupasinghe et al. (2000b) found that 1-MCP did not affect TSS but induced a reduction in volatile production which could negatively impact apple on aroma.

2.2.5 Disorders

General: Stone fruit

CI is caused by exposure of a commodity to temperatures below its optimal range (Kader, 2002). As was found by Taylor et al. (1993b) overripeness of 'Songold' plums is a natural occurrence in the ripening process while GB and IB are abnormal physiological processes which take place when the fruit were stored for more than 30 days at -0.5°C. These low temperatures for too long periods at a time without intermittent warming causes the membranes to become irreversibly permeable thus losing their regulatory ability. Longer periods at -0.5°C also cause an increase in the viscosity of water soluble pectin as well as a decrease in the extractable juice. Thus GB and IB are generally associated with low levels of extractable juice. This is as a

result of the binding of water soluble pectins to water and free ions into gel complexes. This binding occurs when there is an increase in membrane permeability at the stage when the water soluble pectins have a high gel potential.

The incidence of GB in 'Peeka' apricots increased significantly during the ripening period with an increase in the cold storage period at -0.5°C (De Klerk and Von Mollendorff, 1994). In peaches (Ben-Arie and Sonego, 1980) and nectarines (Zhou et al., 1999) CI is known as woolliness, which is very common in South Africa (Eksteen et al., 1986). It is caused by an imbalance of PG and PE caused by prolonged exposure to low temperatures and promotes abnormal degradation of cell wall pectins. This imbalance is caused by the inhibitory effect cold storage places on PG (Von Mollendorff et al., 1992). Furthermore it has also been found that PG is ethylene regulated (Sitrit and Bennett, 1998).

In a storage experiment with nectarines it was found by Zhou et al. (2001), that severity of injury in individual fruits was positively related to inhibition of ethylene. Application of ethylene during storage decreased the percentage of woolly fruits when compared to the control fruit. Similar results were found by Dong et al. (2001a) with experiments on 'Flavortop' nectarines and they found that treatment of the fruit with 1-MCP increased the occurrence of the disorder. The fruit treated with ethylene, the control fruit and fruit treated with 1-MCP had 70%, 20% and 0% healthy fruit, respectively (Dong et al., 2001a).

Delayed storage treatments of two days at 20°C before storage at 0°C almost eradicated CI of nectarines (Retamales et al., 1992; Zhou et al., 2000; Zhou et al., 2001). It was speculated that the delayed storage allowed the initiation of the ripening process to begin and was supported by the fact that the fruit lost no firmness during the two days at 20°C but started softening and generally had higher ethylene production during the storage time at 0°C when compared to the control fruit (Zhou et al., 2001). Furthermore it was proven on an individual fruit basis that healthy fruit displayed a typical climacteric but with increased severity of woolliness ethylene production and respiration rate decreased (Zhou et al., 2000). Thus in nectarines ethylene seems to counteract CI (Zhou et al., 2000).

Controlled atmosphere storage: Stone fruit

It has been demonstrated that CA stored nectarines had a high percentage of sound fruit (Retamales et al., 1992; Zhou et al., 2000). The fruit stored under CA had PG activities similar to the air treated fruit during storage (Zhou et al., 2000). After five days ripening the level of PG activity was higher and PE activity lower than the control fruit resulting in a high PG / PE ratio. Zhou et al. (2000) speculated that a rapid rise in PG activity and a high PG / PE ratio during ripening could play a crucial role in preventing woolliness of nectarines. Thus CA appears to repress PG activity during storage but the fruit retains the ability to recover from the repression when rewarmed (Zhou et al., 2000). Ben-Arie et al. (1993) suggested that the equilibrium between PE and PG in nectarines is renewed due to the inhibitory effect elevated CO_2 places on the activity of PE allowing the fruit to ripen without developing IB.

The storage of nectarines and peaches under CA conditions has shown much promise in the restriction of CI disorders (Crisosto et al., 1997; Ke and Kader, 1992; Levin et al., 1995; Lurie at al., 1993; Zhou et al., 2000). Treatment of nectarines and peaches with low O_2 as a quarantine measure did not damage any of the fruit externally and no internal injury was found after five days of treatment of 'Fire Red' peaches and 'Fantasia', 'Flamekist' and 'Royal Giant' nectarines (Ke et al., 1994). In contrast to this Smilanick and Fouse (1989) found that low O_2 levels of 0.5% caused detrimental changes after just three days, whether at 25°C or at 15°C.

Truter et al. (1994c) found that a CA treatment of 5% O_2 and 19% CO_2 significantly reduced the incidence of GB in 'Peeka' and 'Imperial' apricots. Furthermore high CO_2 treatments had an inhibitory effect on the development of decay in 'Super Gold' and 'Imperial' apricots. Truter et al. (1994c) also found that a high temperature treatment of 20°C with 21% O_2 and 5% CO_2 for two days prior to different lengths of storage at -0.5°C for each cultivar eradicated IB and decay on 'Olympia', 'Zaigina', 'Nectared 9' and 'Flamekist' nectarines. Truter and Combrink (1992) were also able to restrict the onset of overripeness in 'Songold' plums by storage under CA. Truter and Combrink (1997) also found that CA storage of 'Songold' plums for eight weeks (cycles of 10 days at -0.5°C and 18 days at 7.2°C) and seven days at 10°C in air was able to restrict GB (10.9%) while the control fruit were 100% affected.

In contrast to these findings Eksteen and Truter (1986) found that CA stored, South African grown 'Flamekist' nectarines were as susceptible and at times more susceptible to woolliness disorder than fruit not stored under CA. Similarly the breakdown of 'Santa Rosa' plums was not affected by CA storage and at times the incorrect CA conditions (3% O_2 and 4% CO_2) promoted decay (Eksteen and Truter, 1986). They suggested that this increase in decay was possibly due to low O_2 injury.

General: Avocados

Avocado fruit are very sensitive to CI (Pesis et al., 1994). Storage of 'Fuerte' and 'Hass' avocados at 10°C did not cause CI (Eaks, 1976). However, storage at 5°C and 0°C caused CI and the severity thereof increased with longer storage times and lower temperatures (Eaks, 1976). It has also been shown for many plant tissues that ion leakage is a characteristic of CI (Hariyadi and Parkin, 1991; Lafuente et al., 1991; McCollum and McDonald, 1991). Pesis et al. (1994) supported this correlation between ion leakage and CI by prestorage low oxygen treatment of 'Fuerte' avocados. Although the difference was not always significant the fruit treated with low oxygen had reduced CI and lower leakage than the untreated fruit.

It has been found that the sensitivity of 'Fuerte' and 'Hass' avocado to CI is dependant on the stage of the ethylene climacteric (Donkin, 1995). The fruit are less sensitive at the climacteric rise than at the climacteric peak and least sensitive post-climacteric (Donkin, 1995). This is supported by Florissen et al. (1996) who found that increased ethylene levels, during the storage of 'Hass' avocados, made the fruit more susceptible to CI. Due to its role in CI White et al. (2001) recommend that avocados are stored under low ethylene conditions. CI symptoms include: blackening and pitting of the exocarp, grey-brown discolouration of the mesocarp and uneven ripening (Couey, 1982).

Mesocarp discolouration may range from a light grey discolouration at the distal part of the fruit to a complete blackening of the mesocarp (Donkin, 1995; Van Lelyveld and Bower, 1984). This discolouration is not necessarily accompanied by browning of the vascular strands (Donkin, 1995; Van Lelyveld and Bower, 1984). The two

commonly occurring forms of mesocarp discolouration were classified by Swarts (1984) as i) grey pulp: a grey to brown discolouration of the mesocarp, or ii) pulp spot: localised grey spots on the cut surface of the mesocarp associated with the cut ends of vascular bundles. The oxidation of o-diphenols to o-quinones by the enzyme polyphenoloxidase (PPO) results in these browning reactions (Bower and Cutting, 1988). If cell membrane integrity is upset or they rupture, phenolic acids are oxidised to quinones which, once polymerised, produce a brown colour (Torres et al., 1987).

Controlled atmosphere storage: Avocados

CA storage and, to a lesser extent, high CO_2 treatments can increase the shelf life of avocados (Bower, 1986; Truter and Eksteen, 1983; Eksteen and Truter, 1985). However an effective preharvest and postharvest disease control programme should be followed due to the increased susceptibility of the fruit to anthracnose rot with increased shelf life (Bower, 1986; Truter and Eksteen, 1983; Eksteen and Truter, 1985). It was also found that the fruit were less susceptible to CI after treatment with CA or high CO_2 (Bower, 1986; Truter and Eksteen, 1983; Eksteen and Truter, 1985). Eksteen and Truter (1985) further found that to gain the most benefit from CO_2 shock and CA storage the fruit must be exposed to the treatment as soon after harvest as possible. They found less promising results if the fruit were treated even four days after harvest. Exposing avocados to low O_2 levels (nitrogen levels of 97%) for 24 hours also reduced CI after storage at 2°C (Pesis et al., 1993).

Truter et al. (1991; 1992) found that CA (2% O_2 and 10% CO_2) stored 'Fuerte' avocado fruit had the lowest PPO activity during storage but it increased during softening to reach the highest levels when compared to the control and CO_2 shock (25% CO_2, with O_2 decreasing to 1% after three days) treated fruit. However, Truter et al. (1991 and 1992) also found that the CA and CO_2 shock treated fruit generally had fewer internal disorders than the control fruit. This, according to Cutting et al. (1990) is due to a change in activity of PPO just before softening and probably the stage that ripening takes place (Truter et al., 1992). This change in PPO activity occurs because CA storage kept the fruit in the same condition as at harvest (Bower et al., 1990). Therefore the fruit do not display the characteristic decrease in PPO activity during the early stages of softening which fruit stored in air do (Bower et al.,

1990). Therefore during this process the inactivation of PPO results in a lower activity where browning has occurred as opposed to CA where no browning has taken place (Truter et al., 1992). Bower et al. (1989a) confirmed that PPO activity decreased as the fruit ripened. They, however, found a higher PPO activity in the CA stored fruit after storage but also through the ripening period. They confirmed findings by Truter et al.(1992) that the CA stored fruit despite the high PPO activity had fewer internal disorders.

It was shown that exposure of avocado to elevated CO_2 levels before storage increased the level of antifungal diene that makes fruit more resistant to *Colletotrichum gloeosporioides* (Prusky et al., as reported by Lange and Kader, 1997), but the exposure of the fruit to these stress levels may result in some undesirable physiological changes (Lange and Kader, 1997). Yahia and Carrillo-López (as reported by Ke et al., 1995) found that treatment of 'Hass' avocado fruit with 0.1 to 0.4% O_2 and 50 to 75% CO_2 for longer than a day at 20°C before ripening in air caused exocarp and mesocarp injury. On the other hand, 'Fuerte' avocados treated with an initial CO_2 concentration of 5% which increased to 35% CO_2 three days after harvest followed by normal storage prevented anthracnose, CI, grey flesh and pulp spot (Truter and Eksteen, 1987).

Pulp CI of 'Hass' avocado stored at 5°C under a CA of 21% O_2 and 1% CO_2 was present after three weeks storage and increased after five and seven weeks storage (Meir et al., 1995). Meir et al. (1995) found that CA combinations of high CO_2 (8%) and high O_2 (21%) or low CO_2 (0.5%) and low O_2 (3%) during the storage of 'Hass' avocados reduced the incidence of pulp injury due to chilling. Pulp injury was almost completely eradicated by treatment with low O_2 (3%) and high CO_2 (3 to 8%) and fruit were of good quality after nine weeks storage displaying a possible synergistic effect between the two gases on restricting disorder development. Meir et al. (1995) found that the best combination tested for CA storage of 'Hass' avocados was 3% O_2 and 8% CO_2 and suggested that results may be improved by slight increase in temperature to reduce CI further.

1-MCP storage: General

Treatment of 'Flavortop' nectarines with 1-MCP inhibited fruit softening but this may have been related to the increase of storage disorders, viz. flesh woolliness and flesh reddening (Dong et al., 2001a). White et al. (2000) found that treatment of 'Hass' avocados with 1-MCP almost completely removed flesh greying and internal CI while the control fruit were approximately 90% affected. White et al. (2000) also suggested that lower concentrations of 1-MCP (≤ 250 nl.l^{-1}) are optimal otherwise fruit will tend to rot before they ripen.

2.2.6 Respiration

General

Temperature has the main effect on fruit respiration (Hardenburg, 1971) and ripening during storage. This relationship between temperature and ripening is expressed as the temperature coefficient (Q_{10}), which describes the increase in respiration for a 10°C rise in temperature (Kader, 2002). For most non-chilling sensitive commodities an increase of 10°C above the optimum storage temperature will result in a two to three fold increase in respiration and thus deterioration.

Controlled atmosphere storage: General

The rise in respiration during ripening of certain fruit without an increase in the storage temperature was named the respiratory climacteric by Kidd and West (as reported by Blanke, 1991). It is well documented that low O_2 and high CO_2 partial pressures reduce respiration rates of plant material (Beaudry, 1999; Beaudry, 2000; Bhowmik and Pan, 1992; Kader, 1986; Solomos, 1993). Apparently for climacteric tissues this will result in improved storability (Beaudry, 1999).

The use of CA in fruit storage reduces the gradient of CO_2 from the fruit to the ambient atmosphere and therefore causes an accumulation of CO_2 within the fruit (Blanke, 1991). This, according to Blanke (1991), causes a slowing down in activity of malate decarboxylase and of the respiratory enzymes thus slowing down or

retarding the respiratory climacteric. Furthermore the inhibitory effect of CO_2 results from a decreased succinate dehydrogenase activity causing an initial irreversible accumulation of succinate and so suppressing apple respiration (Hulme, as reported by Blanke, 1991; Kader, 1995). 'Bartlett' pears treated with air, air and 5% CO_2, air and 10% CO_2, and air and 20% CO_2 after 4 days at 20°C had respiration rates of 35, 27, 20 and 15 ml $O_2.kg^{-1}.h^{-1}$ (Ong, as reported by Kader, 1989). Thus the decrease in respiration rates and the delay of the onset of the climacteric were proportional to the decrease in O_2 concentration and increase in CO_2 concentration. The same was found in 'Cox's Orange Pippin' (Olsen, 1982) and 'Gala' apples (Solomos, 1993). As O_2 levels in the atmosphere are decreased there is a proportionate decrease in respiration rate but only to a certain degree at which point there will be a shift towards anaerobic respiration. At low O_2 levels, increasing CO_2 to 5% will almost halve the respiration rate of the apples (Olsen, 1982).

Carbon dioxide shock treatments result in a progressive reduction in fruit respiration (Blanke, 1991). This is done by slowing fruit metabolism, mitochondrial activity and respiration after succinate dehydrogenase is inhibited (Blanke, 1991). Furthermore fermentative pathways are induced (Ke et al., 1995). When electron transport and oxidative phosphorylation are inhibited fermentative metabolism's function is to use NADH and pyruvate so that glycolysis can proceed (Ke et al., 1995). Thus via substrate phosphorylation the production of some ATP will be allowed, which will help the plant tissue to survive temporarily as long as the fruit is not exposed to CA stress beyond its limits (Ke et al., 1995).

Storage of 'Fiesta Red' nectarines under 5% CO_2 and 3% O_2 or 20% CO_2 and 15% O_2 significantly repressed respiration without an apparent climacteric rise, when compared to an air treatment (Levin et al., 1995). It was also observed by Eris et al. (1993) that CA storage with 5% O_2 and 5% CO_2 significantly reduced the respiration rate of sweet cherries when compared to the other atmospheres in which the fruit were stored.

Young et al. (1962) found that levels of CO_2 between 5 - 10 % for 21 days depressed respiration of avocado fruit and delayed the climacteric rise in respiration. Kader (1995) found that there was lactate build up in avocado fruit for three days under

exposure to 0.25% O_2 but 80% CO_2 did not have the same effect. Pesis et al. (1994) found that a prestorage low O_2 treatment lowered the respiration rate of 'Fuerte' avocados during storage at 2°C.

1-MCP storage: General

The average respiration rate of pear fruit treated with 280 $nl.l^{-1}$ 1-MCP (15.3 $nmol.kg^{-1}.s^{-1}$) and held in air for three to five days was significantly lower than the control (20.1 $nmol.kg^{-1}.s^{-1}$) (De Wild et al., 1999). An effect on carbohydrate metabolism was expected when banana fruit treated with 1-MCP had respiration rates lower than the control fruit (Golding et al., 1998).

De Wild et al. (1999) also discussed the combined effect of treatment with CA and 280 $nl.l^{-1}$ 1-MCP on pears. They found an insignificant combined effect by this treatment as respiration rate slowed from 12.4 - 10.3 $nmol.kg^{-1}.s^{-1}$ with the addition of the 1-MCP before treatment of the fruit with CA.

2.2.7 Ethylene production

General

With the onset of ripening the sharp increase in climacteric ethylene production is regarded as the control mechanism for changes in colour, texture, aroma, flavour and other physiological attributes (Lelièvre et al., 1997). Thus, ethylene is influential in the developmental processes of fruit ripening and senescence (Dilley et al., 1995).

Ethylene biosynthesis was characterised by Adams and Yang (as reported by Yang, 1985): Meth (Methionine) to SAM (S-adenosylmethionine) to ACC (1-aminocyclopropane-1-carboxylic acid) to ethylene (C_2H_4). There are two main enzymes which are involved:

- ACC synthase catalyses the conversion of SAM to ACC.
- ACC oxidase catalyses the conversion of ACC to ethylene.

The conversion of SAM to ACC is regarded as the rate limiting step (Yang, 1985). This was proven by Cameron et al. (1979) by application of ACC to various plant organs which resulted in a marked increase in ethylene. This indicated that the enzyme converting ACC to ethylene (ACC oxidase) was present in most plant tissue but that ACC synthase was not readily available for conversion of SAM to ACC.

Furthermore, the physiological role of ethylene in fruit ripening can be explained by the model proposed by McMurchie (as reported by McGlasson, 1985). Ethylene biosynthesis is regulated by two systems, viz. system I and system II. System I is present in both mature and immature fruit and is involved in the development of the ACC oxidase activity as well as regulation of the aging process. It is also responsible for low ethylene production rate during growth. System II can only be found in mature climacteric fruit once ripening is initiated. Furthermore it is responsible for the development of the ACC synthase activity. Prior to the climacteric the low levels of ethylene produced by system I together with its receptor are responsible for the breakdown of the inhibitor which inhibits the binding of ethylene to system II. The development of the ACC synthase and the oxidase enzymes results in the autocatalytic increase in ethylene production.

Studies have shown that in pre-climacteric fruit a contributing factor to the low rate of ethylene production is due to ACC malonylation (Lelièvre et al., 1997). This occurs when ACC is converted to malonyl ACC instead of ethylene. This was shown by Golding et al. (1998) by treatment of bananas with 1-MCP before the onset of autocatalytic ethylene production. The climacteric was delayed and malonylation increased.

Controlled atmosphere storage: General

It is generally accepted that the primary mechanism by which CA extends the storage life of apples is by suppression of ethylene biosynthesis (Gorny and Kader, 1996a; Solomos, 1993). Yang (1985) stated that increased CO_2 levels help delay the ethylene ripening action but Kader (1986) found that elevated CO_2 levels can reduce, promote or have no effect on ethylene production depending on the commodity and CO_2 concentration. It has been found that CO_2 at concentrations normally found in

intercellular air spaces during fruit ripening is required to make ACC oxidase catalytically competent so as to convert ACC to ethylene (Poneleit and Dilley, 1993), but at higher concentrations can reversibly inhibit ethylene action (Dilley et al., 1995; Gorny and Kader, 1996b).

It was originally thought that CO_2 was a competitive inhibitor of ethylene action (Burg and Burg, 1967; Salisbury and Ross, 1991b), but as stated earlier CO_2 acts as a non-competitive inhibitor affecting ACC synthase or ACC oxidase (De Wild et al, 1999; Gorny and Kader, 1993). This is supported by the levels of ACC synthase in 'Bartlett' pears treated with air and 1% CO_2 or air and 20% CO_2 (Chavez-Franco and Kader, 1993). Compared to the control, there was a decrease in the enzyme activity as the level of CO_2 increased. In contrast air and 1% CO_2 stimulated ACC oxidase, supporting what was found by John (1997). Bufler (1984) reported that elevated CO_2 levels during storage of apples inhibited ACC synthase and delayed the burst in ethylene production during ripening. The exposure of 'McIntosh' apples to 12% CO_2 for two weeks at either 0 or 3°C under low oxygen followed by CA storage suppressed ethylene evolution (Bramlage et al., 1977).

O_2 levels below 8% decrease ethylene production and the sensitivity to ethylene in fresh fruit and vegetables (Kader, 1986) and levels below 0.25% severely suppress ethylene biosynthesis (Gorny and Kader, 1996b). It has also been shown that O_2 is needed for the production and action of ethylene (Beaudry, 2000; Burg and Burg, 1967). This is because O_2 is required for the conversion of ACC to ethylene (Gorny and Kader, 1996b; Salisbury and Ross, 1991b), and explains why ethylene production is almost halted in hypoxic conditions (Salisbury and Ross, 1991b).

Treatment of 'Gala' apples with decreasing concentrations of O_2 (Solomos and Kanellis, 1989) caused an increasing delay in initiation of ethylene production, and there was no rise in ethylene production in samples kept under 2% O_2. Similarly the ethylene production of banana was strongly related to O_2 concentration and declined substantially at levels below 5% O_2 (Elyatem et al., 1994). Gorny and Kader (1996b) reported that 'Golden Delicious' apples, which had already attained the capacity for system II autocatalytic ethylene production, when treated with air and 20% CO_2 or 0.25% O_2 effectively reduced the ethylene biosynthetic rate.

Controlled atmosphere storage: Stone fruit

According to Abdi et al. (1998) plums can be classed as either climacteric or suppressed climacteric. Suppressed climacteric plums produce a fraction of the ethylene and production starts later when compared to climacteric plums, thus they ripen slower. Kruger et al. (2001) categorised 'Songold' as suppressed climacteric since it produced 20 $\mu l.kg^{-1}.h^{-1}$ ethylene after five weeks storage at -0.5°C and one week of shelf life at 15°C. Since 'Pioneer' produced 140 $\mu l.kg^{-1}.h^{-1}$ it is regarded as climacteric. This genetic trait could possibly be utilised so that ripening restriction could be achieved by closely monitored low temperature storage, thus completely negating the need for CA storage.

For each incremental increase in CO_2 content of the storage atmosphere the ethylene production of 'Fiesta Red' nectarines was further reduced until, at 20% CO_2, the fruit was producing 2% of the ethylene that was produced without CO_2 (Levin et al., 1995). Similar to this low O_2 insecticidal atmospheres had the effect of lower ethylene production as well the accumulation of ethanol and acetaldehyde associated with anaerobic respiration (Smilanick and Fouse, 1989).

Controlled atmosphere storage: Avocados

The climacteric rise associated with avocado fruit ripening is facilitated by detachment from the tree (Blanke, 1991). Avocado fruit attached to the tree have only trace amounts of ACC and once harvested there is a strong increase in ACC synthase activity and thus ACC accumulation (Blumenfield et al., 1986). It was further hypothesised that ACC is the rate limiting step during tree ripening and that this is limited by the low levels of ACC synthase possibly inhibited by a ripening inhibitor (Blumenfield et al., 1986).

It was reported that the climacteric ethylene production of 'Fuerte' avocados was quickly inhibited by 20% CO_2 at 20°C (Cheverry et al., 1988). However, the ACC content of the 'Fuerte' avocados was not modified (Cheverry et al., 1988). Thus it seems that the high CO_2 levels inhibited the conversion of ACC to ethylene (Chavez-

Franco and Kader, 1993; Cheverry, et al., 1988). In contrast to this Van Eeden et al. (1990) found that a 20% CO_2 shock treatment for three days followed by ripening decreased the amount of ACC formed during the climacteric of 'Hass' avocados suggesting that this treatment reduced the fruits ability to produce ACC. Both the findings support the statement by De Wild et al. (1999) that CO_2 is a non-competitive inhibitor of ethylene by affecting either of the two main enzymes involved in ethylene production. Furthermore it has been found that while CA storage extends the storage life of avocados when compared to the control the fruit stored under CA have a lower peak in ethylene production and once exposed to shelf life temperatures will ripen quicker than fruit exposed to the shelf life temperatures directly after harvest (Meir et al., 1998).

Pre-climacteric 'Hass' avocados were stored in air or under elevated CO_2 (20% CO_2 and 17% O_2 or 40% CO_2 and 13% O_2) (Lange and Kader, 1997). On day four all treatments were transferred to air and the fruit stored in air had ethylene production levels of 250 - 350 $\mu l.kg^{-1}.h^{-1}$ by day four and five while the fruit stored under elevated CO_2 levels only started ethylene production on day five reaching $\pm$ 55 $\mu l.kg^{-1}.h^{-1}$ (Lange and Kader, 1997). Ethylene production was completely inhibited while the fruit were stored under the elevated CO_2 levels. Similar to elevated CO_2 conditions Pesis et al. (1994) considered the effect of lowered O_2 conditions as prestorage treatment for 'Fuerte' avocados. It was found that while the control fruit had an increase in ethylene production during storage (2°C) the ethylene production of the treated fruit stayed low. With the increase in temperature to 17°C the climacteric increase of the O_2 treated fruit occurred four days after the untreated fruit.

1-MCP storage: General

Untreated 'Gulfruby' plums and 'Beauty' plums show a typical climacteric but when treated with 1-MCP ripening and ethylene production was delayed for several days (Abdi et al., 1997). This was also apparent in the suppressed-climacteric 'Shiro', 'Rubyred' and 'Red Rosa' plums (Abdi et al., 1997; Dong et al., 2001b). Similar results were found on 'Hass' avocados treated with 30 $nl.l^{-1}$ 1-MCP or higher when stored at 22°C (Feng et al., 2000). The subsequent treatment with 300 $ul.l^{-1}$ ethylene

for 24 hours had no effect and the peak in ethylene production was delayed by 12 - 13 days (Feng et al., 2000). The results were the same when pears were treated with 280 $nl.l^{-1}$ 1-MCP (De Wild et al., 1999). Banana fruit stored at 20°C and treated with 1-MCP displayed a suppression in the climacteric but the maximum ethylene production was also enhanced (Golding et al., 1998).

De Wild et al. (1999) investigated the combined effect of treatment with CA and 280 $nl.l^{-1}$ 1-MCP on pears. The ethylene production was significantly less in the combined treated fruit (29.8 $pmol.kg^{-1}.s^{-1}$) compared to the fruit treated only with CA (52.7 $pmol.kg^{-1}.s^{-1}$) and only with 1-MCP (57.8 $pmol.kg^{-1}.s^{-1}$).

2.3 Modified atmosphere packaging (MAP)

MAP is commonly utilised to allow development and / or maintenance of atmospheres other than air with the use of polymeric films (Kader and Watkins, 2000). An advantage of the use of MAP is that the time in storage is usually shorter for cut produce (minimally processed) leading to shorter exposure times to injurious atmospheric levels (Kader and Watkins, 2000). Additionally, due to the high value of the produce, temperature control is more consistent (Kader and Watkins, 2000). Furthermore it has been found that seal packaging of citrus with high-density polyethylene at 20°C reduced weight loss more than cooling of the fruit at the lowest possible temperature without injury occurring (Ben-Yehoshua et al., 1981).

If the product and film permeability characteristics match properly with a package the desired modified atmosphere can be generated passively via the respiration of the product through O_2 consumption and CO_2 production (Kader and Watkins, 2000). To maintain the desired atmosphere within the package the permeability of the package must allow O_2 to enter at a rate offset by the consumption of O_2 by the product, and similarly CO_2 should be vented out of the package. The atmosphere within the package can also be set actively by replacing the atmosphere with the desired gas mixture (Kader and Watkins, 2000). Further control can be applied with the use of ethylene absorbers to prevent the climacteric rise in ethylene (Kader and Watkins, 2000), carbon dioxide absorbers to prevent excessive build up of CO_2 within the

package (Kader and Watkins, 2000) and oxygen absorbers to accelerate O_2 depletion (Furlani et al., 1993).

Further advantages of MAP were highlighted by Kader and Watkins (2000):

- Maintain high relative humidity and so reduce water loss.
- Reduced contamination of products during handling.
- Spread of decay between produce is reduced.
- The film can carry fungicides, absorbers or other chemicals.
- Information to the customer can be displayed on the packaging.

There are several reasons which restrict the use MAP:

- Atmosphere cannot be accurately adjusted within the package to account for temperature fluctuations which can result in anaerobic conditions due to higher respiration rates of the fresh produce (Clarke and DeMoor, 1997).
- Expenses for the modification of packaging lines to accommodate MAP and MAP technology on the basis of film cost, makes it difficult to justify its use (Kader and Watkins, 2000).
- Package integrity must be able to withstand normal handling operations during transport (Kader and Watkins, 2000).

2.4 Relative humidity

2.4.1 General

Relative humidity (RH) control forms a integral part of storage of fresh produce. It is an environmental factor which influences rates of water loss of whole plants or excised plant organs (Forney and Brandl, 1992). Many physiological processes are affected by the rate of water loss such as cell expansion, growth, photosynthesis and senescence (Forney and Brandl, 1992). RH should be kept high in order to reduce moisture loss, skin shrivel and softening (Miller and Risse, as reported by Polderdijk et al., 1993). The problem, however, is that RH is very temperature sensitive and any fluctuations in temperature at high RH could result in condensation on the fruit, a condition favourable for soft rot development organisms.

The difficulty in controlling and measuring RH over the years has meant that it is an inconvenience and its role in the physiology of plants has generally been ignored (Gaffney, 1978). According to Solomon different salts solutions can be used to control humidity but a different salt is needed for each humidity and the RH varies with temperature (Forney and Brandl, 1992). Equilibrium RH can also be formed with nonsaturated solutions such as glycerol (Forney and Brandl, 1992). Glycerol solutions are easy to mix, the exact composition can be determined and it is relatively inexpensive (Forney and Brandl, 1992).

At an RH of 97% a fluctuation in temperature of as little as 0.25°C can cause precipitation (Meredith as reported by Grierson and Wardowski, 1978). For this reason Hardenburg et al. (1986) recommend RH ranging from 85% to 95% for storage of fresh produce. This presents a balance between microbial spoilage and mass loss (Shirazi and Cameron, 1992). Shirazi and Cameron (1992) identified a number of compounds which could be used in MAP to control humidity: sorbitol, xylitol, NaCl, KCl and $CaCl_2$. Ten grams of each compound with a mature green tomato resulted in stable RH of 75%, 80%, 75%, 85% and 35%, respectively while the control packages ranged between 96 - 100% RH throughout the experiments.

It was suggested by Ben-Yehoshua et al. (1983) that sealed packages which increase the RH of the environment delay senescence and retain firmness of fruit and vegetables by alleviating water stress.

2.4.2 Mass loss

Water loss during postharvest handling of mango fruit will reduce the market quality (Macnish et al., 1997). Similarly, stone fruit (Mitchell, 1986; Rushing and Dinamarca, 2000) will develop shrivel due to water loss thus also decreasing market quality. Plums generally lose water less easily than other stone fruit (Mitchell, 1986). The amount of water movement will depend on surface characteristics of the fruit, RH, air velocity and temperature (Mitchell as reported by Crisosto, 1995).

Storage temperature has a large influence on mass loss of fruit. The lower mass loss at lower temperatures can be explained as a lower vapour pressure difference between the fruit and the atmosphere at lower temperature. This effect has been observed in stone fruit (Mitchell, 1986) including cherries (Maguire, 2001). By lowering the temperature of the environment fruit temperature is also reduced thus lowering the partial pressure of water vapour at the fruit surface (Maguire, 2001). In other words cold storage reduces the driving force for water loss. Increased RH in storage also reduces the driving force for water loss (Maguire 2001; Hatfield and Knee, 1988).

Increasing RH during storage significantly reduced moisture loss of litchi fruit (Jiang and Fu, 1999), bell peppers (Ben-Yehoshua, et al., 1983; Lurie et al., 1986; Polderdijk et al., 1993), 'Kensington Pride' mangos (Macnish et al., 1997), avocados (Bower et al., 1989b; Littmann, 1972; Meir et al., 1998), 'Stark Red Gold' nectarines (Verstreken and De Baerdemaeker, 1994), pears (Littmann, 1972), bananas (Littmann, 1972) and mature green tomatoes (Bhowmik and Pan, 1992). It must be born in mind that mass loss is a combination of both moisture loss (transpiration) and dry mass loss (respiration) (Bhowmik and Pan, 1992). Therefore under CA storage, with lower respiration rates, mass loss is less. Transpiration loss is, however, a greater contributor to total mass loss than respiratory losses.

2.4.3 Colour development and firmness

Colour development of 'Kensington Pride' mangos (Macnish et al., 1997) and bananas (Littmann, 1972; Xue et al., 1995; Xue et al., 1996) did not progress as strongly at high RH levels (95%) compared to lower RH levels.

The correlation between high humidity storage, membrane integrity and retention of firmness was strongly linked to the water status of the fruit (Ben-Yehoshua et al., 1983; Lurie et al., 1986). This was proven by the fact that there was less water in the peel of 'Eureka' lemons stored at lower relative humidity (Ben-Yehoshua et al., 1983). Similarly the firmness losses of bell peppers (Ben-Yehoshua et al., 1983; Lurie, et al., 1986; Polderdijk et al., 1993), citrus fruit (Ben-Yehoshua et al., 1979; Ben-Yehoshua et al., 1983), 'Kensington Pride' mangos (Macnish et al., 1997), 'Giant Cavendish' bananas (Xue et al., 1995; Xue et al., 1996), 'Stark Red Gold' nectarines

(Verstreken and De Baerdemaeker, 1994) and mature green tomatoes (Bhowmik and Pan, 1992) was restricted at high RH levels of ± 95%. Xue et al. (1995) stated that increased PG activity was positively correlated to the loss in flesh firmness at low RH levels.

2.4.4 Disorders

Prevention of the development of pitting and other blemishes of cucumbers can be achieved by restricting water loss from the chilling-sensitive tissues by high RH levels (Chien et al., 1998). Furthermore Haard and Hultin (1969) observed that abnormalities in banana fruit ripening after storage at an RH below 80% were very similar to the abnormalities associated with chilled fruit. Due to RH levels nearing 100% during the storage of the 'Red Bell' peppers (Polderdijk et al., 1993) the level of decay was increased but RH levels as high as 90% during storage of litchis (Jiang and Fu, 1999) restricted browning of the pericarp, anthocyanin loss as well as reduced activity of PPO resulting in far improved fruit quality. Similarly the incidence of pathological and physiological disorders of 'Fuerte' avocados were decreased by decreasing the water stress on the fruit (Bower et al., 1989a; Bower et al., 1989b). This was coupled to a higher PPO activity in the water stressed fruit. However, it was found that this decreased water stress had no effect on the susceptibility to external CI (Bower et al., 1989b).

Moderate RH during MA storage of mangos was found to reduce red spot CI at 12°C (Pesis et al., 2000) but levels nearing 95% promoted the disorder. Therefore, RH should be at a level so as to reduce water loss but at the same time the air should not be close to water saturation otherwise decay and other disorders could be promoted (RH of ± 95%) (Pesis et al., 2000). Sodium chloride was used as the hygroscopic material during storage of 'Red Bell' pepper to lower the in package humidity (88 - 97% RH) (Rodov et al., 1995). This reduction in RH was able to reduce decay and thus extend life of the fruit when compared to fruit sealed without the hygroscopic material.

By lowering RH during the storage of apples the likelihood of cellular bursting would be reduced and thus reducing the appearance of CI symptoms (Bramlage, 1982). It

was concluded, however, that regardless of the mechanism by which lowered RH reduces CI in apples it is not desirable due to increased mass loss and the possibility of shrivel (Bramlage, 1982).

2.4.5 Respiration, ethylene production and fruit ripening

It was demonstrated by Littmann (1972) that the shelf life of pears, bananas and avocados can be extended by reducing the rate of postharvest water loss. This was achieved by increasing the RH level in the storage environment and Littmann (1972) speculated that this restricted the onset of ethylene production. Therefore Littmann (1972) showed that there is a significant relationship between moisture loss and time to onset of the climacteric. At low RH (4%) the percentage loss of green life for pears was 30.2%, avocados was 40% and bananas was 20% when compared to fruit stored under higher RH (95%) (Littmann, 1972).

MA storage of non-waxed mangos under low RH increased respiration and ethylene levels and so fruit ripened faster (Pesis et al., 2000). 'Fuerte' and 'Hass' avocados were stored under very low RH, by passing dry air over the fruit (10 - 20% RH), and high RH by passing moist / wet air over the fruit (90 - 95% RH) (Adato and Gazit, 1974). Carbon dioxide and ethylene levels were monitored in the storage environment and kept at a low level so that the fruit were purely influenced by the change in RH. The avocados lost water about three times faster in the dry air treatment than the wet and consequently the rate of ripening was hastened by approximately 30% (Adato and Gazit, 1974). These findings were supported by experiments on 'Fuerte' avocados (Meir et al., 1998) and 'Giant Cavendish' bananas (Xue et al., 1996).

It was found that storage of bell peppers under high RH had no influence on CO_2 production but markedly slowed the deterioration processes which occurred in the normally stored fruit (Lurie et al., 1986). In non-climacteric fruit, which do not undergo as much ripening after harvest, ripening processes were found to be more inhibited by the storage environment RH than by storage temperature. Thus it was suggested that water stress may trigger senescence (Lurie et al., 1986). Storage of citrus fruit under high RH with the use of a skin coating or film delayed the

deterioration of the fruit by restricting water stress and not by the change in internal O_2, CO_2 and ethylene concentrations (Ben-Yehoshua, 1969; Ben-Yehoshua et al., 1979).

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3. ARTICLE 1: Regular and Controlled Atmosphere Storage of 'Songold' and 'Angeleno' Plums under Different Temperature Regimes

REGULAR AND CONTROLLED ATMOSPHERE STORAGE OF 'SONGOLD' AND 'ANGELENO' PLUMS UNDER DIFFERENT TEMPERATURE REGIMES

Abstract

Historically a dual temperature shipping regime was used for the export of South African plums. This previously used dual temperature (DT) regime (10 days at -0.5°C and eight days at 7.5°C) was compared in season one to a single high temperature (7.5°C) for 18 days with and without controlled atmospheres on 'Songold' and 'Angeleno' plums. Fruit were then transferred to 10°C for seven days to simulate shelf life. Both controlled atmospheres (CA1 and CA2) at each temperature regime delayed ripening of the fruit in terms of flesh / skin colour and firmness. 'Songold' fruit stored at 7.5°C and at DT under CA had firmness values of ± 4.0 kg and ± 4.4 kg, respectively while the firmness value of the air treated fruit at each temperature were 2.5 kg and 3.1 kg, respectively, after 18 days storage. 'Songold' fruit stored in air, showed an increase in respiration (CO_2 production) during the storage period regardless of temperature regime with the fruit stored under DT peaking higher than the fruit stored at 7.5°C at ± 19.8 $mg.kg^{-1}.h^{-1}$ on day 14. 'Angeleno' fruit stored at 7.5°C under CA2 and fruit stored under DT in air had the highest incidence of decay and aerated flesh (23.0% and 76.0%, respectively) while fruit stored at 7.5°C under CA1 had the lowest levels of decay (3.0%). 'Angeleno' fruit stored at 7.5°C and DT in air also showed an increase in respiration during storage with a similar peak of ± 18.5 $mg.kg^{-1}.h^{-1}$ on day 17. Only 'Angeleno' fruit stored at 7.5°C in air produced ethylene (± 1.2 $\mu l.kg^{-1}.h^{-1}$). During season two a more realistic storage time frame of 43 days was adopted which involved storage from the date of harvest. This would include transport to the harbour (six days), the shipping period (18 days) and storage time to the market (19 days). This was followed by six days shelf life at 15°C. The fruit were stored under either a long (18 days at 7.5°C) or a short dual temperature regime (six days at -0.5°C and 12 days at 7.5°C) during the shipping time with or without CA. Both the temperature regimes stored with CA had positive results in delaying colour development and softening of the fruit. All the fruit displayed strong increases in respiration rate and ethylene production rate with the increase in temperature to 15°C. Gel breakdown was prominent through all the

treatments of the 'Songold' experiment while aerated flesh was very prominent in the 'Angeleno' experiment. Storage of these two cultivars at higher temperatures with CA showed much promise when compared to the commercially used storage method. However, these treatments should be attempted on climacteric plum cultivars which display stronger ethylene production and ripening and which should gain more benefit from higher temperature storage in combination with CA.

Introduction

Although ±0°C is recommended for storage of plums in the USA (Hardenburg et al., 1986; Mitchell, 1986), Chile, Israel and Australia, South Africa has developed a dual temperature shipping regime (type of intermittent warming) for some cultivars to reduce internal breakdown, a chilling injury disorder (Boyes and De Villiers, as cited by Taylor, 1996). Since 'Songold' plums were found to be susceptible to this disorder (Eksteen, 1982), the dual temperature regime was adopted as it was found that internal breakdown could be prevented for this cultivar (Hartmann et al., 1988). However, the dual temperature regime was not able to solve the problem of gel breakdown (GB), which was possibly unmasked with the application of this temperature regime. GB is the gelatinous breakdown of the mesocarp tissue between the stone and the middle of the mesocarp (Taylor et al., 1993).

The dual temperature shipping regime allows fruit to be stored from the time of harvest at -0.5°C for approximately 10 days as it was found that longer time caused chilling injury. Thereafter the fruit would be held for the remainder of the shipping period at 7.5°C. Therefore the dual temperature shipping regime would have ± 10 days at -0.5° and 8 - 18 days at 7.5°C.

According to De Swart and Redelinghuys (1968), between 11.4 and 20.3% of all rejections on plum fruit between 1962 and 1967 could be ascribed to green and overripe fruit. They attributed this to incorrect picking maturities as well as storage at unsuitable temperatures, which were the main causes for cold storage disorders in plums. Factors such as the fruits ability to develop high eating quality, susceptibility to mechanical injury, postharvest performance and potential postharvest life is

determined by stone fruits maturity at harvest (Crisosto et al., 1995). It was shown by Abdi et al. (1997) that GB was most severe when 'Radiant', 'Gulfruby' and 'Shiro' plums were harvested after they were considered to be commercially mature (3.0 kg). The same was found by De Swart and Redelinghuys (1968), who added that these advanced maturities left fruit vulnerable to decay, overripeness and bladderiness.

Research protocol at the beginning of the 2001/2 season specified the use of a maximum 36 day storage period for early South African plum cultivars and a maximum 43 day storage period for later plum cultivars. Whether early or late, this protocol recommended a dual temperature storage regime during the shipping period. Late season fruit, would be held at -0.5°C until reaching the harbour (about four days). Once CA treatment and shipment starts fruit would be held at -0.5°C for a further six days. Thereafter temperatures would be increased to 7.5°C for the remainder of the shipping period (± 12 days). During storage and transport from the harbour to the market once overseas, fruit would be held at -0.5°C (± 21 days). Once at the market a variable shelf life time and temperature can be assumed (five to seven days, 15 - 20°C).

The recommended atmospheres for storage of plums are 1-2% O_2 and 0-5% CO_2 (Kader, 1997). Eksteen et al. (1986) found that O_2 levels between 2-6% and CO_2 levels between 2-10% on 'Santa Rosa' and 'Songold' plums stored at -0.5°C had no effect on controlling internal breakdown, extending the cold storage period or maintaining fruit quality over a storage period of three to six weeks. Truter et al. (1994a), however, found that 'Songold' plum fruit stored for seven weeks under intermittent warming performed best under CA conditions of 3% O_2 and 5% CO_2 for the full storage time. This was opposed to storage under partial CA of 2% O_2 and 5% CO_2 for the first 10 days of storage after which the fruit were held under regular atmosphere conditions. Only 4.5% GB occurred after seven weeks of CA storage, while control fruit showed decay after five weeks. 'Casselman' plum fruit stored under CA reacted similarly to the 'Songold' plums, having 3.4% GB and firmness of 5.1 kg. Truter et al. (1994a) also investigated high temperature conditioning (storage at 20°C under a gas mixture of 21% O_2 and 5% CO_2 for two days and afterwards seven weeks of intermittent warming under regular atmosphere conditions), CA storage (3% O_2 and 5% CO_2) and partial CA storage of 'Laetitia' plums for eight

weeks and a further week at 10°C shelf life temperature. After eight weeks storage the fruit had no internal breakdown.

Kader (1997) recommended a shipping / storage temperature of 10°C with the use of CA. However, South Africa decided to use 7.5°C since it was successful in decreasing internal breakdown and because export shipping periods are longer than those used in the USA. We hypothesised that plum fruit could be stored under CA at a single high shipping temperature (7.5°C) and that this would compare favourably with the current commercial dual temperature shipping regime. It would also allow fruit to be harvested more mature, thereby enhancing eating quality.

Materials and Methods

Season 1:

Experimental set up: 'Songold' and 'Angeleno' plums were harvested at Sandrivier farm between Paarl and Wellington (Lat: 33.0°S and Long: 19.6°E) on the 17th February and 12th March 2001, respectively. Both harvests were relatively late so as to harvest more mature fruit. The fruit were held at -0.5°C for two to three days before being arranged into the different treatments. All damaged fruit were discarded. The experiment consisted of two temperature regimes. The dual temperature regime was the commercial shipping temperature of 10 days at -0.5°C and eight days at 7.5°C (DT). The single high temperature regime involved keeping the fruit at 7.5°C for the entire 18 days (time periods were taken from the time of arrival at the laboratory). In addition, three atmospheres were used at each temperature, namely an air control, controlled atmosphere one (CA1) and controlled atmosphere two (CA2).

The air supply was humidified and supplied via flow boards. In the case of the controlled atmospheres, the flow rates were ± 150 ml.min^{-1} governed by glass capillaries and the air treatment had flow rates of ± 400 ml.min^{-1} governed by needle valves. The CA composition was checked regularly and maintained within 10% of the required concentrations using an O_2 / CO_2 analyser (PBI-Dansensor, Combi Check 9800-1, Ringsted, Denmark). The experiment was a 2 x 3 factorial design with six replicates per treatment. Thus, the entire experiment had six treatments with six

buckets (replications) per treatment. After 18 days of storage, fruit were kept for seven days at 10°C to simulate shelf life.

Expt 1: 'Songold'

A representative set of 60 fruit (ie. six replicates of 10 fruit) was taken initially and evaluated prior to the fruit being put under atmosphere. Thereafter, 10 fruit per bucket were removed for evaluation after 18 days of storage at either 7.5°C or DT, and another 10 fruit after the seven days of shelf life at 10°C.

Colour. The fruit were evaluated for external colour at each of the three evaluation times (initially on arrival, after storage, after shelf life at 10°C). A colorimeter (NR-3000, Nippon Denshoku, Tokyo, Japan) was used for the colour evaluation at a point on the fruit where colour was most uniform. The colorimeter gives values for chroma, lightness and hue angle (°).

Firmness. After the colour readings were taken the fruit were evaluated for firmness at the three dates. Readings were taken on peeled, opposite cheeks of the fruit with a penetrometer (Southtrade fruit pressure tester, FT 327, Alphonsine, Italy) fitted with an 11 mm tip.

Total soluble solids (TSS). The same fruit were evaluated for TSS at the three dates. A slice from each side of each fruit within a replicate was juiced together and a TSS reading taken using a hand held refractometer (Atago PR-100 9501, Japan).

Mass loss. At the start of the experiment a group of five fruit per bucket was placed in a punnet in the buckets separate from the rest of the fruit, and their mass was recorded at each evaluation time to calculate mass loss.

Respiration. CO_2 levels were measured with the use of an infra-red gas analyser (IRGA) (Infra-Red Gas Analyser, S151, Kingston, Ontario), which was connected to the out flow from each of the buckets. Readings were taken approximately every third day during the 18 day storage time, but only on the fruit stored in air, as the

controlled atmospheres had CO_2 levels greater than 0.2% (2000 $\mu l.l^{-1}$) which is the upper limit of the IRGA. Hence the flow rates of the air treated fruit were higher to allow CO_2 to be measured.

Ethylene production. Gas samples were taken from the out flow of each bucket four times during the 18 day storage period and analysed for ethylene by gas chromatograph (GC Series 3000, Varian 4290 integrator, Varian Instrument Group, Palo Alto, California).

Internal Ethylene content (IEC). A partial vacuum was applied on individual fruit with the use of a glass vacuum container with a gas tight lid and a vacuum pump (Saltveit, 1982). Within the container the fruit was held in a flask filled with water with a septum at the point were the gas from the fruit accumulates when the vacuum is applied. After the vacuum had been applied and released a sample of the extracted gas was taken with a gas tight syringe and evaluated using a gas chromatograph. After 18 days of storage, two fruit per replication were removed and evaluated, and another two fruit per replication evaluated after the seven days shelf life period.

Statistical Analysis. Analysis of variance (ANOVA) of the main effects and LSD values with a significance level of 5% were obtained using Statistical Analysis Systems (SAS). Presented data points are the means of the six replications ±SE.

Expt 2: 'Angeleno'

As with the 'Songold' experiment, fruit were analysed initially, after 18 days storage and after a further seven days of shelf life. Since there were fewer fruit available only 30 fruit (ie. six reps of five fruit) were used per treatment per evaluation time, thus a representative set of 30 fruit were evaluated before the experiment started.

Measurements of firmness, TSS, mass loss and internal ethylene content were taken as described for the 'Songold' experiment. Since the skin colour of 'Angeleno' plums does not change with maturity, flesh colour was measured. The stem end of the fruit above the pip was sliced off and a reading taken on the sliced section on a point where

colour was the most uniform and not influenced by the pip. Colour was measured after 18 days storage and after seven days of shelf life.

Titratable acidity (TA). Juice obtained for TSS was also analysed for TA by titrating a pooled juice sample with 0.1 N NaOH to a pH of 8.2 using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland). Results were expressed as percent malic acid.

Disorders. Fruit were rated for internal browning (IB), GB, aerated flesh (air filled cells in mesocarp give fruit a dull appearance) and overripeness, as described by Taylor (1996) as well as decay. These ratings were done as a percentage of the five fruit evaluated per replication.

Respiration and ethylene production. Respiration readings were taken every third day during the 18 day storage period but only on the air treated fruit. During the seven days shelf life period half the replications were left on a regular atmosphere flow board (flow rate: ± 400 $ml.min^{-1}$) so that respiration and ethylene production could be measured daily for all the treatments.

Statistical Analysis. Analysis of variance (ANOVA) of the main effects and LSD values with a significance level of 5% were obtained using Statistical Analysis Systems (SAS). Presented data points are the means of the six replications ±SE.

Season 2:

Experimental set up: 'Songold' and 'Angeleno' plums were received from Colors export company on the 12th February 2002. The fruit were harvested on the 7th February and 6th February 2002, respectively. Due to an uncommon summer rainy season in the Western Cape most plum cultivars were harvested approximately two weeks earlier than normal. Both experimental harvests were relatively late, thereby harvesting more mature fruit.

It was decided to follow a new protocol for the storage of plums, to more closely simulate commercial conditions. For late season cultivars, this involved storage for 49 days including a shelf life period. 'Angeleno' plums were held at -0.5°C for

seven days and 'Songold' plums for six days before being arranged into the different treatments. All damaged fruit were discarded. The experiment consisted of two temperature regimes. Short dual temperature regime (short DT) is the commercial shipping temperature regime of six days at -0.5°C and 12 days at 7.5°C. The long dual temperature regime (long DT) involved keeping the fruit at 7.5°C for the entire 18 days of shipping. In addition two atmospheres were used at each temperature regime, namely an air control and controlled atmosphere (CA2).

The air supply was humidified and supplied via flow boards. The flow rates were ± 450 $ml.min^{-1}$ governed by glass capillaries. The atmosphere composition was checked regularly and maintained within 10% of the required concentrations using an O_2 / CO_2 analyser (Dual gas analyser, ICA 15, Tyler House, Tonbridge). The experiment was a 2 x 2 factorial design with four replications per treatment. Thus, the entire experiment had four treatments with four buckets (replications) per treatment. After 25 days of storage since harvest for 'Angeleno' and 24 days for 'Songold' the 'Angeleno' plum fruit were kept for 18 days and 'Songold' plum fruit for 19 days at -0.5°C. Thereafter the fruit were kept for six days at 15°C to simulate shelf life.

A representative set of 10 fruit was taken initially and evaluated prior to the fruit being put under atmosphere. Thereafter, 10 fruit per bucket were removed for evaluation after 24 days storage for 'Songold' and 25 days storage for 'Angeleno', after 43 days storage for both cultivars and again after six days of shelf life at 15°C.

As in season one the fruit were evaluated for colour, firmness, TSS, TA and mass loss. Respiration was measured approximately every second day on the air treated fruit during storage and on the CA treated fruit every second day during storage once removed from CA. All treatments were evaluated daily during the shelf life period. Ethylene production was measured for all treatments every second day during storage and daily during the shelf life period. Internal ethylene was measured as in season one. One fruit per replication was measured at each evaluation time.

Statistical Analysis. Analysis of variance (ANOVA) of the main effects and LSD values with a significance level of 5% were obtained using Statistical Analysis Systems (SAS). Presented data points are the means of the four replications ±SE.

Results

Season 1

Expt 1: 'Songold'

Colour. From the initial hue angle reading (96.0°) 'Songold' fruit stored under DT in CA2 (92.2°), DT in air (89.8°) and at 7.5°C in air (90.5°) became more yellow (hue angle decreased) compared to fruit in the remaining three treatments, which had changes in hue angle of less than 0.5° after the 18 days storage (Fig. 1). After the further seven days at 10°C the fruit stored under DT were all less yellow than fruit stored at 7.5°C. There was a significant difference in hue angle between CA treated fruit stored under DT (± 87.0°) and at 7.5°C (± 81.0°). CA treated fruit at 7.5°C were very similar over the 25 days, regardless of specific atmospheric composition and were significantly yellower than fruit stored under DT in air.

Firmness. The initial firmness of the fruit was 4.6 kg ie. fairly mature (commercial maturity guidelines fall between 4.5 - 7.5 kg). After the 18 days storage the air stored fruit at 7.5°C (2.5 kg) and DT (3.2 kg) were significantly softer than CA stored fruit (4.0-4.8 kg) (Fig. 2). Softening of the CA stored fruit was retarded by the DT regime, in comparison to fruit stored at 7.5°C, however, only fruit stored under DT under CA2 were significantly firmer (4.8 kg).

After a further seven days at 10°C the air stored fruit did not show a large change in firmness when compared to the CA stored fruit regardless of temperature regime. Once again, as with colour, the CA stored fruit at 7.5°C (± 3.2 kg) had fruit firmness values similar to DT stored fruit in air (3.0 kg). CA stored fruit under DT (± 3.6 kg) were the significantly firmest.

Total soluble solids (TSS). TSS values of fruit changed by less than 1% from the initial value of 14.9% (Table 1). After 18 days storage there were no significant differences between the treatments. After a further seven days at 10°C, fruit stored under CA1 at 7.5°C had the significantly highest TSS value (15.0%).

Mass loss. Since shrivel can be a problem on plums, mass loss data are used to evaluate water loss. Significantly highest cumulative mass loss was measured in fruit stored at 7.5°C in air (1.98%) (Table 2). Fruit stored under CA1 at 7.5°C had 1.59% mass loss with the DT stored fruit under CA having the lowest mass loss (± 1.17%). There was no significant difference between CA2 fruit stored at 7.5°C and the DT stored fruit in air.

Respiration. The respiration rate of fruit stored in air at 7.5°C increased steadily from about 9.2 $mg.kg^{-1}.h^{-1}$ on day four to approximately 15.6 $mg.kg^{-1}.h^{-1}$ after 17 days of storage (Fig. 3). Initially, fruit stored under DT in air had a much lower respiration rate (3.8 $mg.kg^{-1}.h^{-1}$ on day 4) but reached a peak of 19.8 $mg.kg^{-1}.h^{-1}$ after 14 days and decreased to about the same level as the fruit stored in air at 7.5°C after 17 days.

Ethylene production. The only fruit which produced measurable ethylene during storage were those stored in air, regardless of temperature regime (Fig 4). Detectable levels were only measured on the penultimate day of the 18 day storage period.

Internal ethylene content (IEC). The IEC of the fruit stored for 18 days in CA were significantly lower than those fruit stored in air (Fig. 5). The fruit stored at 7.5°C in air had significantly highest IEC (4.8 $\mu l.l^{-1}$).

Effects of the DT regime seem to have been overcome during the seven days at 10°C since the IEC increased dramatically during this time. However, the effects of the CA were still present (Fig. 5). The 7.5°C fruit stored in air had significantly higher IEC (20.3 $\mu l.l^{-1}$) than the CA stored fruit regardless of temperature regime.

Expt 2: 'Angeleno'

Colour. After 18 days of storage the fruit flesh colour of the CA stored fruit under DT were significantly less yellow than the remaining treatments (± 81.5°) (Table 3). There was no significant difference between the remaining treatments (73.2° - 74.1°).

After a further seven days at 10°C the fruit stored in CA under DT had flesh colour which were the least yellow (ie. higher hue angle values: CA1 - 78.3° and CA2 - 76.5°) with no significant difference between the two (Table 3). The air stored fruit showed the largest decrease in hue angle at the second evaluation time, with fruit stored under DT in air having significantly yellower flesh colour than the fruit stored under CA, regardless of temperature regime. The fruit stored in CA1 at 7.5°C had little change in colour from the first to the second evaluation date. There was no significant difference between the CA1 fruit stored at 7.5°C and the DT fruit stored under CA.

After 18 days of storage the CA stored fruit under DT had significantly more intense chroma than the remaining treatments (± 41.7) (Table 3). After a further seven days at 10°C the chroma differences between the treatments were insignificant. Fruit stored in air and CA at 7.5°C and under DT in air showed very limited change in chroma over the seven days at 10°C.

Fruit stored in CA under DT were significantly darker than the rest of the treatments after 18 days storage (± 26.6) (Table 3). Although fruit stored at 7.5°C in air or CA2 were significantly lighter than fruit stored under CA1 at 7.5°C and fruit stored under DT in air, their values all fell within about two units. After a further seven days at 10 °C fruit stored under DT in CA were significantly lighter than the air stored fruit, regardless of temperature regime. Although there were significant differences, the remaining treatments all fell within four units (Table 3).

Firmness. The initial firmness of the fruit was 6.0 kg ie. fairly mature (commercial maturity guidelines fall between 4.5 - 8.5 kg), and this decreased to 4.5 kg for fruit stored in air at 7.5°C and was significantly softest after 18 days of storage (Fig. 6). There was no significant difference between the remaining treatments at this stage.

After a further seven days at 10°C there was no significant difference between the fruit stored at 7.5°C in air (3.9 kg) and CA (± 4.3 kg). The fruit stored at 7.5°C in CA were significantly less firm than the fruit stored under DT in CA (± 5.2 kg).

Total soluble solids (TSS). The initial TSS level was 14.7% (Table 4). After 18 days of storage there were significant, albeit small, differences in TSS levels between the treatments. Fruit stored at 7.5°C in air had significantly lower TSS content (14.0%) than all the treatments except the fruit stored under DT under CA2 (14.3%). After a further seven days at 10°C the TSS levels for all the treatments decreased except in fruit stored under DT in CA2 and all values were within less than 1%.

Titratable acidity (TA). All the treatments had similar or higher TA than the initial TA (0.74%) after 18 days storage (Table 4). The fruit stored under storage CA1 under DT had a significantly lower TA level (0.74%) than fruit stored at 7.5°C in air (0.82%) and fruit stored under DT in air (0.82%). After a further seven days at 10°C, TA levels were not significantly different between the treatments (0.77 - 0.83%).

Disorders. There was no occurrence of GB or IB throughout the experiment. Aerated flesh occurred in relatively high quantities after 18 days of storage (Table 5). The CA stored fruit, apart from the fruit stored under CA1 at 7.5°C, all had aerated flesh levels equal to or higher than the fruit stored in air regardless of temperature regime. The fruit stored under CA2 under DT had the highest quantity of aerated flesh (60.0%). All the treatments except fruit stored under CA2 under DT, had an increase in aerated flesh after seven days shelf life. Fruit stored under CA1 under DT had 80.0% aerated flesh while both the CA stored fruit at 7.5°C were fractionally lower (76.7%). Fruit stored under CA2 under DT and at 7.5°C in air fell in the same region (± 45.0%).

Although there was no significant difference between treatments, the level of decay after the shelf life period was high in most of the treatments (Table 5). The fruit stored at 7.5°C under CA1 was the lowest with 3.3%. The remaining fruit all had at least 10.0% decay with the fruit stored at 7.5°C under CA2 and the fruit stored under DT in air the highest at 23.0%.

Mass loss. After 18 days of storage the fruit stored at 7.5°C under CA2 had the significantly highest mass loss (0.60%) (Table 6). Once the fruit entered the shelf life period where all the fruit were moved to 10°C there was no significant difference in moisture loss over those seven days. When considering the cumulative mass loss the determining factor was the 18 day storage where the treatments were held at different temperature regimes. The highest mass loss was measured in the fruit stored at 7.5°C under CA2 (1.05%). This was, however, only significantly higher than the fruit stored under DT under CA1 (0.56%).

Respiration. The respiration rate after three days of the fruit stored at 7.5°C in air was higher (11.5 $mg.kg^{-1}.h^{-1}$) compared to the fruit stored under DT in air (4.8 $mg.kg^{-1}.h^{-1}$), which at that stage were being stored at -0.5°C (Fig. 7). On transfer of the fruit stored under DT to 7.5°C (day 8) the respiration rate of the fruit stored under DT and 7.5°C in air increased and both reached a peak on day 17 at ± 18.4 $mg.kg^{-1}.h^{-1}$. Once the fruit were moved to 10°C, CA treatments ceased and respiration rates were measured on all treatments. Fruit that had been stored at 7.5°C tended to have higher respiration rates than fruit stored under DT. Within each temperature regime, respiration rates were higher in air than CA, although this trend is not as clear for the fruit stored under DT. From day 19 onwards the respiration rate of the fruit stored at 7.5°C under CA fluctuated between the respiration rate of the fruit stored at 7.5°C in air and the respiration rate of the fruit stored under DT in air (± 14 $mg.kg^{-1}.h^{-1}$). The fruit stored under DT under CA had respiration rates which fluctuated below that of the fruit stored under DT in air at about 11 $mg.kg^{-1}.h^{-1}$.

Ethylene production. The fruit stored at 7.5°C in air was the only treatment which displayed any meaningful ethylene production, reaching maximum ethylene production on day 21 at 1.2 $\mu l.kg^{-1}.h^{-1}$ (Fig 8). The further increase in ethylene production was thought to be because of decay. The fruit stored at 7.5°C under CA1 did have one day where there was ethylene production on day 24 (0.6 $\mu l.kg^{-1}.h^{-1}$). The rest of the treatments had no measurable ethylene production.

Internal ethylene content (IEC). After 18 days of storage the fruit stored at 7.5°C in air had significantly highest IEC (1.5 $\mu l.l^{-1}$) (Fig 9). The remaining treated fruit all had IEC less than 0.5 $\mu l.l^{-1}$. The fruit stored under DT in air had 0.5 $\mu l.l^{-1}$ IEC while

the fruit stored at 7.5°C under CA were both in the region of ±0.2 $\mu l.l^{-1}$ IEC. The CA treated fruit all had significantly lowest IEC after storage.

After a further seven days at 10°C all the fruit had an increase in IEC. The fruit stored at 7.5°C in air had the significantly highest IEC (5.5 $\mu l.l^{-1}$). There was no significant difference between the remaining treated fruit and values ranged between 0.5 - 1.1 $\mu l.l^{-1}$.

Season 2

Expt 1: 'Songold'

Colour. After 24 days of storage most of the treated fruit showed a lowering in all three colour parameters from the initial evaluation (chroma: 36.2; lightness: 60.2; hue: 102.5°) (Fig. 10, 11 and 12). This evaluation time showed very similar patterns for all three colour parameters. The fruit stored under long DT under CA had the highest values for chroma and lightness (chroma: 36.9 and lightness: 59.0) although not significantly higher than the fruit stored under short DT under CA (chroma: 34.7 and lightness: 58.1) and vice versa for hue angle (long DT under CA: 96.7° and short DT under CA: 98.4°). The fruit stored under long DT in air had the significantly lowest chroma and lightness (chroma: 31.7; lightness: 55.9), but the hue angle was not significantly lower than the hue angle of the fruit stored under short DT in air (88.9° and 90.7°, respectively).

After 43 days of storage a similar trend developed between treatments when compared to 24 days of storage. There was little change in colour of the fruit over the time period at -0.5°C. The CA treated fruit had the significantly highest values for all three colour parameters regardless of temperature regime, while there was no significant difference between the air treated fruit with regard to hue angle regardless of temperature regime.

The shelf life period saw stronger colour development with regard to all three colour parameters, as the fruit were held for six days at 15°C. There was no significant difference in chroma, lightness and hue angle between the CA treated fruit and no

significant difference between the air treated fruit, regardless of temperature regime. The CA treated fruit saw a drop in hue angle of ± 6.0° to ± 89.3° during the six days at 15°C, regardless of temperature regime. The air treated fruit showed a sharper drop in hue angle of ± 9.0° to ± 81.7°, regardless of temperature regime.

Firmness. Starting with an initial firmness of 4.0 kg the most softening was recorded in the fruit stored under long DT in air after 24 days storage (2.2 kg) (Fig. 13). The least softening occurred in the fruit stored under short DT under CA (3.7 kg). This was, however, not significantly firmer than the fruit stored under long DT under CA (3.4 kg). As with the colour readings, there was very little change in firmness for all the treated fruit between the first (24 days) and second evaluation (43 days) while the fruit was held at -0.5°C. The CA treated fruit were significantly firmer than the air treated fruit regardless of temperature regime. There were no significant differences between the temperature regimes under CA.

During the six days at 15°C all the treated fruit softened. The fruit stored under long DT in air were significantly softest (1.0 kg) while the fruit stored under long DT under CA were firmest (1.8 kg). This was, however, insignificantly firmer than the fruit stored under short DT under CA.

Total soluble solids (TSS). For the duration of the experiment there was no more than a 1% change in the TSS of any of the treated fruit (Table 7). After 24 days storage there was no significant difference between the treated fruit. Most of the values were slightly higher than the initial reading of 13.4%. After 43 days storage, the fruit stored under short DT in air had significantly lower TSS (13.3%) than the fruit stored under long DT under CA which had the highest TSS but there was no significant difference between the remaining fruit. After the six days at 15°C the fruit stored under long DT under CA had the highest TSS (14.1%) although not significantly higher than the fruit stored under short DT in air (13.7%).

Titratable acidity (TA). From the initial TA level of 1.46% all the treated fruit had a decrease in TA after 24 days storage (Table 7). The fruit stored under short DT under CA had the significantly higher TA (1.34%) compared to the fruit stored under

long DT in air (1.22%) which had the lowest TA level. After 43 days storage the fruit stored under short DT under CA had the highest TA (1.26%) together with the fruit stored under long DT under CA (1.23%). This was, however, only significantly higher than the fruit stored under long DT in air. After a further six days at 15°C there was no significant difference between the treated fruit with TSS values ranging from 1.03 - 1.11%.

Disorders. IB occurred at low levels and there was no significant difference between the treatments (Table 8). The fruit stored under short DT in air had the highest incidence of IB (3.1%). GB was significantly highest in the fruit stored under long DT in air (44.4%). The remaining treatments had no significant difference in GB levels although levels as high as 13.9% were recorded in the fruit stored under long DT under CA. The presence of slight decay was observed after the six days at 15°C. However, there were no significant differences between treatments.

Mass loss. After 24 days storage the fruit stored at short DT under CA had significantly lower moisture loss (0.01%) than the fruit stored under long DT in air (0.10%) (Table 9). During the time which the fruit was held at -0.5°C, there was no significant difference in moisture loss between the different treatments, with values ranging from 0.24 - 0.39%. After the six days at 15°C the fruit stored under short DT under CA had the significantly least moisture loss (0.09%) while there was no significant difference between the remaining treatments. Subsequently, the fruit stored under short DT under CA had significantly lower cumulative mass loss (0.41%) when compared to the fruit stored in air under long DT (0.83%) and short DT (0.84%).

Respiration. The first respiration readings were taken on the 11th day after harvest (Fig. 14). The fruit stored under long DT in air were respiring at 14.4 $mg.kg^{-1}.h^{-1}$ while the fruit stored under short DT in air were respiring at 4.0 $mg.kg^{-1}.h^{-1}$. With the increase to 7.5°C for the short DT regime on day 12, the respiration rate of the fruit stored under short DT in air climbed to fluctuate slightly below the levels of the fruit stored under long DT in air at 16.4 $mg.kg^{-1}.h^{-1}$ on day 15. At the end of the shipping

period (day 24) and the subsequent drop in temperature to -0.5°C there was a drop in respiration rate for both the air treated fruit, regardless of temperature regime.

At this point (day 24) the CA treatments came to an end and the first readings for the CA treated fruit were taken on day 26. During the time period at -0.5°C respiration in the fruit from both CA treatments fluctuated at ± 2.9 $mg.kg^{-1}.h^{-1}$, slightly below the levels of the air treated fruit, regardless of temperature regime (± 5.5 $mg.kg^{-1}.h^{-1}$).

On day 43 the shelf life period at 15°C started. This produced a sharp increase in respiration rate for all the fruit. The respiration of the air treated fruit increased to ± 25.0 $mg.kg^{-1}.h^{-1}$ regardless of temperature regime, and fluctuated at approximately that level for the shelf life duration at 15°C. The respiration rate of the CA treated fruit increased to ± 19.5 $mg.kg^{-1}.h^{-1}$ regardless of temperature regime. However, values did not fluctuate at that level but continued to increase slowly without slowing by the end of the shelf life period (± 23.2 $mg.kg^{-1}.h^{-1}$).

Ethylene production. The first signs of ethylene production were observed in the fruit stored under long DT in air on day 15 (Fig. 15). The ethylene production fluctuated, reaching levels no higher than 1.5 $\mu l.kg^{-1}.h^{-1}$ up until the shelf life period. The fruit stored under short DT in air were the only other fruit to produce measurable ethylene during the storage time. These fluctuated slightly lower than the fruit stored under long DT in air reaching levels no higher than 0.8 $\mu l.kg^{-1}.h^{-1}$.

With the increase in temperature to 15°C all the fruit had an increase in ethylene production. The fruit stored under long DT in air were the only fruit to reach a peak in ethylene production (6.0 $\mu l.kg^{-1}.h^{-1}$), on day 48. Ethylene production continued to increase in all the remaining treated fruit through the duration of the shelf life period with the fruit stored under short DT in air reaching the highest ethylene production levels (9.7 $\mu l.kg^{-1}.h^{-1}$) on day 49.

Internal ethylene content (IEC). At the first evaluation time on day 24, the fruit stored under long DT in air had the significantly highest IEC (50.9 $\mu l.l^{-1}$). The two CA treatments had the lowest IEC regardless of temperature regime (± 0.6 $\mu l.l^{-1}$),

although not significantly lower than the fruit stored under short DT in air (Fig. 16). At the second evaluation time on day 43, the fruit stored under long DT in air had a lower, although still significantly highest IEC (32.8 $\mu l.l^{-1}$). All the remaining fruit had an increase in IEC. The greatest increase was in the fruit stored under short DT in air, which increased by ± 21.3 $\mu l.l^{-1}$ to 27.1 $\mu l.l^{-1}$. The CA treated fruit didn't show as large an increase, reaching ± 1.7 $\mu l.l^{-1}$ and had the significantly lowest IEC, regardless of temperature regime.

At the third evaluation time on day 49, the largest increases were in the CA treated fruit. The fruit stored under short DT under CA reached 122.1 $\mu l.l^{-1}$ and were significantly higher than the fruit stored under long DT under CA, which reached 74.6 $\mu l.l^{-1}$. The highest IEC at the third evaluation was in the fruit stored under short DT in air (214.1 $\mu l.l^{-1}$). This was, however, not significantly higher than the fruit stored under long DT in air (185.3 $\mu l.l^{-1}$).

Expt 2: 'Angeleno'

Colour. After 25 days of storage there was a drop in all the colour parameters from the initial readings (chroma: 33.7; lightness: 64.3; hue angle: 92.4°) (Table 10). With regard to chroma, the fruit stored under long DT in air was the highest (32.6) although, only significantly higher than the fruit stored under short DT in air (31.2). There were no significant differences in lightness values between any of the treated fruit at the first evaluation time. The fruit stored under long DT in air had the significantly lowest hue angle (82.4°) and there was no significant difference between the remaining treated fruit.

During the storage at -0.5°C there was a slight increase in value from the first evaluation time for all the colour parameters except the chroma in the fruit stored under long DT in air, which showed a slight decrease and was subsequently the significantly lowest (31.9). Both the air treated fruit had significantly lowest hue angle (long DT: 90.3°; short DT: 91.0°). The CA treated fruit had significantly highest values for all the colour parameters, regardless of temperature regime.

During the shelf life period there was a decrease in value for all the colour parameters relative to the second evaluation. The fruit stored under long DT in air had the significantly lowest lightness (46.6) and hue angle (65.3°). The CA treated fruit had the significantly highest lightness (long DT: 55.9; short DT: 55.8) and hue angle (long DT: 87.8°; short DT: 85.6°), regardless of temperature regime. The fruit stored under short DT under CA had significantly higher chroma (30.7) than the air stored fruit under short DT (28.0) and long DT (26.9).

Firmness. After 25 days of storage all the fruit softened from the initial firmness value (6.3 kg) (Fig. 17). The fruit stored under short DT under CA showed the least softening and was subsequently the significantly firmest (6.2 kg) by almost a kilogram of firmness from the fruit stored under long DT under CA. Both the air treated fruit were significantly softest (± 4.5 kg), regardless of temperature regime.

On day 43 after the storage period at -0.5°C the fruit stored under short DT in air showed little change (4.5 kg) while the remaining treatments all softened. The fruit stored under long DT in air were the significantly softest (3.6 kg) while the remaining treatments had no significant difference in firmness.

The six days at 15°C resulted in a further softening of all the treated fruit with the fruit stored under long DT in air significantly softest (2.3 kg). There was no significant difference between the CA treated fruit stored under short DT (4.0 kg) and long DT (3.8 kg). Likewise, there was no significant difference between the fruit stored under long DT under CA and the fruit stored in air under short DT (3.6 kg).

Total soluble solids (TSS). At all three evaluation times there was no significant difference in TSS content between any of the treated fruit (Table 11). Values ranged between 12.2% - 12.7% throughout the experiment.

Titratable acidity (TA). The TA values decreased from the initial value of 1.36% after 25 days storage (Table 11). The fruit stored under short DT under CA had significantly higher TA (1.30%) than the fruit stored under short DT in air (1.17%). After 43 days storage there was a further drop in TA levels with no significant

difference between treatments (± 1.08%). After a further six days at 15°C the CA treated fruit had the significantly highest TA levels regardless of temperature regime (long DT: 1.20% and short DT: 1.24%).

Disorders. IB only occurred in the fruit stored under long DT and there were no significant differences between the fruit stored under CA (17.0%) and the fruit stored in air (11.1%) (Table 12). Although statistically insignificant, aerated flesh was more prominent in the short DT treated fruit under CA (25.7%) than under short DT in air (19.4%). The fruit stored under long DT in air had the lowest incidence of aerated flesh (5.6%). The fruit stored under long DT under CA were the only fruit which had decay (2.8%). Subsequently there was no significant difference between the treatments.

Mass loss. After 25 days of storage there was no significant difference in mass loss between any of the treated fruit (Table 13). The fruit stored under long DT under CA had the lowest mass loss (0.23%) and values ranged up to 0.37% in the remaining fruit. With the storage time at -0.5°C there was less mass loss. The fruit stored under long DT in air had significantly higher mass loss (0.26%) than the fruit stored under short DT in air (0.04%).

After the shelf life period at 15°C there was no significant difference in mass loss between any of the treated fruit. The fruit stored under long DT in air had significantly higher cumulative mass loss (0.89%) than the CA treated fruit, regardless of temperature regime (long DT CA: 0.49%; short DT CA: 0.54%).

Respiration. On day 12 when the first respiration readings were taken the fruit stored under short DT in air had a lower respiration rate (3.9 $mg.kg^{-1}.h^{-1}$) than the fruit stored under long DT in air (9.5 $mg.kg^{-1}.h^{-1}$) (Fig. 18). On day 13 with the increase in temperature to 7.5°C, for the fruit stored under short DT, the respiration rate of the fruit stored under short DT in air increased and fluctuated at a level much the same as the fruit stored under long DT in air to the end of the shipping period (± 11.5 $mg.kg^{-1}.h^{-1}$).

With the decrease in storage temperature to -0.5°C and the ending of the CA treatments the air treated fruit showed a drop in respiration rate to ± 3.2 $mg.kg^{-1}.h^{-1}$, which fluctuated for the remaining time at ± 4.0 $mg.kg^{-1}.h^{-1}$ regardless of temperature regime. The CA treated fruit performed much the same, fluctuating slightly lower than the air treated fruit at ± 3.2 $mg.kg^{-1}.h^{-1}$, regardless of temperature regime.

For all fruit the increase in temperature to 15°C resulted in a large increase in the respiration rate on day 43, followed by a slight decrease. The fruit stored under long DT in air reached the maximum respiration rate (22.3 $mg.kg^{-1}.h^{-1}$) on day 48. The fruit stored under short DT in air had a slightly lower maximum respiration rate (20.9 $mg.kg^{-1}.h^{-1}$) on day 44. The CA treated fruit had a very similar reaction reaching maximum respiration rate at ± 17.8 $mg.kg^{-1}.h^{-1}$ on day 44.

Ethylene production. The first evidence of ethylene production was in the fruit stored under long DT in air on day 12 (0.3 $\mu l.kg^{-1}.h^{-1}$) but was not detectable on day 13 (Fig. 19). Thereafter the fruit stored under long DT in air and the fruit stored under short DT under CA had momentary ethylene production on day 40 (± 0.1 $\mu l.kg^{-1}.h^{-1}$). On day 47, during the shelf life period, the fruit stored under long DT in air had a strong increase in ethylene production reaching the highest level of all the fruit on day 49 (2.1 $\mu l.kg^{-1}.h^{-1}$). The fruit stored under short DT in air first produced measurable ethylene on day 48, reaching 0.6 $\mu l.kg^{-1}.h^{-1}$ on day 49. The fruit stored under long DT under CA showed a slight peak in ethylene production on day 48 (0.3 $\mu l.kg^{-1}.h^{-1}$).

Internal ethylene content (IEC). After the shipping period, on day 25, the fruit stored under long DT in air had significantly higher IEC (0.7 $\mu l.l^{-1}$) than the CA treated fruit which had the significantly lowest IEC regardless of temperature regime (Fig 20). After 43 days storage, all the fruit had an increase in IEC. At this time the fruit stored under long DT in air had the significantly highest IEC (6.2 $\mu l.l^{-1}$) while the CA treated fruit had significantly lowest IEC (long DT CA: 0.7 $\mu l.l^{-1}$; short DT CA: 0.5 $\mu l.l^{-1}$). After the shelf life period the fruit stored under long DT in air further increased in IEC (7.6 $\mu l.l^{-1}$) and was significantly highest. The remaining treated fruit had no significant difference and showed a decrease in IEC during the shelf life period.

Discussion and Conclusion

Colour. Hue angle is regarded as the most representative parameter for the change in colour of fruit. For both seasons and both cultivars, colour development generally followed the temperature profile. When held at low temperatures, colour development was retarded while at higher temperatures and especially during the shelf life periods, colour development was accelerated. Kruger et al. (2001) found that 'Songold' fruit stored at -0.5°C showed faster ripening upon exposure to warmer temperatures than fruit not exposed to the lowered temperatures. This was not apparent in the 'Songold' experiments in this study, but did appear in the season one 'Angeleno' experiment where the DT stored fruit in air had the most rapid colour development during the shelf life period.

During season one the combination of the DT regime with CA had the effect of slowing colour development even further. The combination of CA treated fruit stored at a single high temperature regime followed closely, with the DT fruit stored in air. In season two, with the longer storage time, the CA treated fruit had slower colour development than the fruit stored in air, regardless of temperature regime. This is because CA conditions highly impact colour changes during ripening and senescence, particularly the changes from green to yellow (Beaudry, 2000; Kader, 1986; Mattheis and Fellman, 2000). The colour changes generally associated with the ripening of 'Wickson' plums were delayed by storage under 30, 50 and 75 kPa O_2 and ripened at 20°C (Claypool and Allen, as reported by Kader and Ben-Yehoshua, 2000). Similarly, CA storage restricted the green colour loss of 'Golden Delicious', 'Granny Smith' and 'Starking' apples when compared to the control fruit (Truter et al., 1982).

Firmness. The effects of CA on the fruit could be seen clearly in firmness. The air stored fruit, regardless of temperature regime, softened more rapidly during storage and ripening. Polygalacturonase (PG) and pectinesterase (PE) are the key enzymes involved in the hydrolysis of cell wall pectin (Dong et al., 2001). In 'Flavortop' nectarines it has been found that PG activity was very low and PE activity high at harvest and the reverse occurred as fruit ripened (Dong et al., 2001). Fruit softening is regarded as the ripening process most sensitive to ethylene (Lelièvre et al., 1997) and it has been found that PG is ethylene regulated (Sitrit and Bennett, 1998).

Therefore, the fact that increased CO_2 levels affect both of the enzymes involved in ethylene production (De Wild et al., 1999) goes a long way to explaining why CA storage restricts fruit softening, as has been found with experiments on plums (Rushing, 1993), nectarines (Levin et al., 1995; Lurie et al., 1993; Rushing, 1993; Truter et al., 1994b), peaches (Rushing, 1993), apples (Knee, as reported by Kader, 1986; Truter et al., 1982) and pears (Truter, 1987; Truter, 1990)

In season one, the fruit stored under DT in air ripened slower than fruit stored at 7.5°C in air while in season two the fruit stored under short DT in air was significantly firmer at all the evaluation times than the fruit stored under long DT in air. This relationship between temperature and ripening is expressed as the temperature coefficient (Q_{10}), which describes the increase in respiration for a 10°C rise in temperature (Kader, 2002). For most non-chilling sensitive commodities an increase of 10°C above the optimum storage temperature will result in a two to three fold increase in respiration and thus deterioration. Kruger et al. (2001) found that softening of 'Songold' plums during shelf life was enhanced by longer preceding storage periods at -0.5°C. In contrast during both season one and two of our experiments the temperature regime under which the fruit was exposed to the longer time at -0.5°C displayed less softening after the shelf life period. This is supported by Mitchell (1986) who found that 'Sungrand' nectarines lost significantly more firmness when stored at 5°C as opposed to storage at 2.2°C.

Total soluble solids (TSS). During the 'Songold' experiment in season one fruit stored at 7.5°C under CA and during season two the fruit stored under long DT under CA had the significantly highest TSS after the shelf life period. According to Goodenough and Thomas (as reported by Kader, 1986), CA conditions slowed down the losses in sugars and organic acids in tomatoes during storage at 12.5°C for up to two months. Similarly CA storage has shown positive results in retaining TSS in plums (Streif, 1989). In contrast to this Levin et al. (1995) found that changing the storage atmosphere had no effect on soluble solid content of 'Fiesta Red' nectarines.

Titratable acidity (TA). During season one TA levels remained similar for all the fruit. During season two, however, the 'Angeleno' plums treated with CA displayed a slower loss in acidity during the time at 15°C. This is in agreement with Kader

(1986) who found that CA storage reduces losses in acidity in fresh fruits. Lau and Looney (1982) found that a 2.5% CO_2 atmosphere in air maintained a higher titratable acidity in 'Golden Delicious' apples. Similar results were found with work on 'Starking' and 'Granny Smith' apples (Eksteen and Truter, 1986; Truter et al., 1982) as well as 'Winter Nelis' and 'Forelle' pears (Truter, 1990).

Disorders. During season one aerated flesh was very prominent throughout all treatments of the 'Angeleno' experiment. It was, however, a season which was prone to aerated flesh and this is seen by the far lower levels during season two, despite the fruit stored under short DT displaying high incidences. According to Kruger et al. (2001) the longer the time spent at -0.5°C, the higher the incidence of disorders. This is not in agreement with Taylor et al. (1994) who found that GB formed more rapidly in 'Songold' plums stored under DT compared to single temperature storage (-0.5°C). However, the development of GB was eventually higher in the single temperature regime stored fruit after a ripening period at 10°C. This indicates that symptom development is suppressed at lower temperatures, despite the fact that physiological injury has occurred.

During season two, the prevention of IB in the 'Songold' fruit was evident. However, GB was prominent in all the treatments but most evident in the fruit stored under long DT in air. GB levels were lower in the CA treated fruit. Taylor et al. (1993) showed that the DT regime led to the development of or unmasking of GB in 'Songold' fruit, although it was able to restrict IB development.

Historically the DT regime was employed to restrict the development of internal breakdown. This was successful, as can be seen from the season two experiments. As already mentioned, the DT regime was used as internal breakdown was prominent at the single low temperature storage (Boyes and De Villiers, as cited by Taylor et al., 1996). However in the 'Angeleno' experiment in season two, IB was only evident in the fruit stored under long DT while during season one IB did not occur in 'Angeleno'. Thus it is possible that the 18 days of storage at -0.5°C after the shipping time during season two had a negative influence on the fruit stored under long DT, causing IB to occur. Alternatively, the extended storage time at -0.5°C for the short

DT stored fruit subsequent to harvest served as a type of conditioning, thereby helping the fruit to cope with the 18 days at -0.5°C and preventing IB.

Chilling injury, as internal breakdown is, is caused by exposure of a commodity to temperatures below its optimal range (Kader, 2002). Zhou et al. (1999) linked the occurrence of woolliness in nectarines, a chilling injury disorder, to an imbalance in PG and PE by prolonged exposure to low temperature. This leads to the abnormal degradation of cell wall pectins. Furthermore, it was found that the severity of injury was directly linked to the inhibition of ethylene and application of ethylene decreased the amount of woolly fruit compared to the control (Zhou at al., 2001). This was backed up by the fact that 1-methylcyclopropene, an ethylene inhibitor, had the opposite effect (Dong et al., 2001). Application of CA to nectarines repressed PG activity during storage but PG retained the ability to recover when the fruit were rewarmed, thus renewing the equilibrium between PG and PE and preventing woolliness (Zhou et al., 2000). It was also suggested that the inhibitory effect that CO_2 has on PE activity allows the fruit to ripen normally (Ben-Arie et al., 1993). CA storage reduces chilling injury of plums (Truter et al., 1994a), nectarines (Levin et al., 1995; Ke and Kader, 1992; Zhou et al., 2000) and apricots (Truter et al., 1994b).

Mass loss. The generally lower mass loss of fruit stored under DT during season one can be explained as a lower vapour pressure difference between the fruit and the atmosphere at lower temperature. This effect has been observed in stone fruit (Maguire et al., 2001; Mitchell, 1986). By lowering the temperature of the environment, fruit temperature is also reduced, thereby lowering the partial pressure of water vapour at the fruit surface. In other words, cold storage reduces the driving force for water loss. Thus the DT treated fruit had a lower partial pressure of water vapour at the fruit surface than the fruit stored at 7.5°C, hence leading to lower levels of water loss. Plums generally have slower water loss than other stone fruit and visual shrivel only appears after 4 to 5% water loss (Mitchell, 1986). During season two there was not much difference in mass loss between the long and short DT stored fruit. This was possibly because the temperature regimes differed only slightly. The CA treated fruit did, however, have less mass loss. It must be born in mind that mass loss is a combination of both moisture loss (transpiration) and dry mass loss (respiration). Therefore, under CA, with lower respiration, mass loss is less.

Moisture loss is, however, a greater contributor to total mass loss than respiratory losses. Despite the fact that the flowrates of the fruit stored in air during season one were different to the fruit stored under CA mass loss was not a problem for both seasons as shrivel did not occur (data not shown).

Respiration. As mentioned earlier the relationship between respiration rate and temperature is known as the temperature coefficient (Q_{10}) (Kader, 2002). This relationship was evident in all the experiments as the respiration of the fruit followed the changes in temperature throughout storage.

The effect of the CA on fruit respiration was most clearly seen during season two when the fruit were held at -0.5°C after shipping. For both cultivars, the respiration rate of the CA treated fruit was lower than the air treated fruit. The rise in respiration rate during ripening of certain fruit without an increase in the storage temperature was named the respiratory climacteric by Kidd and West (as reported by Blanke, 1991). The use of CA causes an accumulation of CO_2 within the fruit by reducing the gradient of CO_2 from the fruit to the ambient atmosphere (Blanke, 1991). This causes a slowing down in activity of malate decarboxylase and of some respiratory enzymes, thus slowing down or retarding the respiratory climacteric (Blanke, 1991). Furthermore, CO_2 inhibits succinate dehydrogenase activity, causing an initial irreversible accumulation of succinate and suppression of apple respiration (Hulme, as cited by Blanke, 1991). This further agrees with Young et al. (1962), who found that storage of avocado fruit in air containing 5 - 10% CO_2 for 21 days depressed respiration rate and delayed the onset of the climacteric. 'Bartlett' pears treated with air, air and 5% CO_2, air and 10% CO_2 and air and 20% CO_2 after four days at 20°C, had respiration rates of 35, 27, 20 and 15 ml $O_2.kg^{-1}.h^{-1}$, respectively (Ong, as reported by Kader, 1989). Thus, the decrease in respiration rates and the delay of the onset of the climacteric were proportional to the decrease in O_2 concentration and increase in CO_2 concentration.

Ethylene production. The fruit stored under CA in our experiments generally had lower ethylene production rates compared to the fruit stored in air, regardless of temperature regime. This was most noticeable during the CA treatment but the 'Angeleno' fruit treated with CA had ethylene production rates still lower than the

fruit stored in air after the shelf life period. It was originally thought that CO_2 acted as a competitive inhibitor of ethylene action but subsequently De Wild et al. (1999) have proven that it acts more as a non-competitive inhibitor of ethylene action affecting the two main enzymes involved in ethylene production.

O_2 levels below 8% decrease ethylene production and the sensitivity to ethylene in fresh fruit and vegetables (Kader, 1986). Both the controlled atmospheres used in the experiments had less than 8% O_2. Burg and Burg (1967) also showed that O_2 is needed for the production and action of ethylene. This was further demonstrated by treatment of 'Gala' apples with air, 8%, 6%, 4% and 3% O_2 (Solomos and Kanellis, 1989). The rise in ethylene production started after 19, 27, 48, 67 and 97 days, respectively, and there was no rise in ethylene production in samples kept under 2% O_2. Thus, as would be expected in our experiments, no significant ethylene production was found in the controlled atmosphere treated fruit throughout storage. However, detectable ethylene levels were measured during the shelf life period.

During season one the fruit stored in air at 7.5°C and season two fruit stored under long DT in air were generally the earliest to start ethylene production, and also reached the highest levels. In contrast Kruger et al. (2001) found that 'Songold' fruit stored at -0.5°C for 10 days started ethylene production seven days into the shelf life period at 15°C. The ethylene levels reached as high as 4 $\mu l.kg^{-1}.h^{-1}$ nine days later. Fruit treated with no cold storage only started ethylene production on the 25^{th} day of storage at 15°C.

According to Abdi et al. (1998) plums can be classed as either climacteric or suppressed climacteric. Suppressed climacteric plums produce a fraction of the ethylene and production starts later when compared to climacteric plums, and they subsequently ripen slower. Kruger et al. (2001) categorised 'Songold' and 'Angeleno' as suppressed climacteric since the fruit produced 20 $\mu l.kg^{-1}.h^{-1}$ and 5 $\mu l.kg^{-1}.h^{-1}$ of ethylene, respectively, after five weeks storage at -0.5°C and one week of shelf life at 15°C. Since 'Pioneer' produced 140 $\mu l.kg^{-1}.h^{-1}$ and did not require the cold storage period it is regarded as climacteric. The same was evident in both season one and two of our experiments where ethylene production rates for 'Songold' never

exceeded 10 $\mu l.kg^{-1}.h^{-1}$ while 'Angeleno' reached no higher than 2 $\mu l.kg^{-1}.h^{-1}$. Thus both cultivars are regarded as suppressed climacteric types.

Internal ethylene content (IEC). The effects of the higher CO_2 levels coupled with the lowered O_2 levels can be clearly seen in most cases where the CA treated fruit had lower IEC than the respective air treated fruit. The effect of the different temperature regimes can be seen by the fact that the fruit stored under DT in air during season one and short DT during season two produced lower levels of IEC when compared to the fruit stored at 7.5°C during season one and long DT in season two in air.

During season one storing 'Songold' and 'Angeleno' plums at 7.5°C in conjunction with CA had positive results in delaying ripening of the fruit without them being exposed to the low, injurious temperatures used in the DT regime. Most noticeable is the positive influence on delaying colour development of 'Songold' and softening of the fruit of both the cultivars, especially in the shelf life period. In both experiments the fruit stored at 7.5°C in air had higher moisture losses. However, in combination with CA they showed slightly less moisture loss.

At the conclusion of season one, focus moved to the prevention of the development of disorders as well as slowing ripening in conjunction with CA at different temperature regimes. Most importantly, this was investigated over a time frame more representative of the commercial situation of harvest to sale in approximately seven weeks. The suppressed climacteric traits of both the cultivars in combination with storage under long DT under CA showed much promise in delaying softening and colour development for the full seven week period as well as restricting GB development in the 'Songold' plums. Importantly, with regard to most of the maturity parameters, the CA treated fruit under long DT stored as well or better than the commercial DT storage method in both the 'Songold' and 'Angeleno' experiments.

However, research which led to the uncovering of the suppressed climacteric traits of certain plum cultivars, such as 'Songold' and 'Angeleno', could in future prove to be more effective a method to exploit for extended storage. The correct temperature regime applied to these suppressed climacteric cultivars could negate the necessity of CA. The use of CA incorporated with a temperature regime with less time spent at

-0.5°C could prove to be more effective in delaying ripening of climacteric plum cultivars.

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Table 1

Total soluble solids (TSS) of 'Songold' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmospheres (CA). Measurements were taken initially, after 18 days of storage, and after a further seven days of shelf life at 10°C.

	Atmosphere		After storage	After shelf life at 10°C
			TSS %	
Initial		14.9		
7.5°C	Air		15.0 ns[z]	14.2 b
	CA1		15.1	15.0 a
	CA2		14.8	14.2 b
DT	Air		15.0	14.2 b
	CA1		15.0	14.0 b
	CA2		15.1	14.3 b
LSD			*0.3268*	*0.6597*

[z] Means separation within columns using least significant differences (0.05)

Table 2

Mass loss (%) and cumulative mass loss (%) of 'Songold' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmospheres (CA). Measurements were taken after 18 days of storage and after a further seven days of shelf life at10°C.

	Atmosphere	After storage	During shelf life at 10°C	Cumulative mass loss (%)
		Mass loss (%)		
7.5°C	Air	0.65 a[z]	1.34 a	1.98 a
	CA 1	0.35 b	1.24 a	1.59 b
	CA 2	0.38 b	0.82 b	1.20 c
DT	Air	0.39 b	0.89 b	1.27 c
	CA 1	0.41 b	0.72 b	1.13 c
	CA 2	0.30 b	0.92 b	1.22 c
LSD		*0.1455*	*0.2374*	*0.3040*

[z] Means separation within columns using least significant differences (0.05)

Table 3

Colour (chroma, lightness, hue angle) of 'Angeleno' plum fruit flesh stored at 7.5°C for 18 days or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmospheres (CA). Measurements were taken after 18 days of storage and after a further seven days of shelf life at 10°C.

	Atmosphere	After storage	After shelf life at 10°C	After storage	After shelf life at 10°C	After storage	After shelf life at 10°C
		Chroma		Lightness		Hue angle (°)	
7.5°C	Air	27.3 c[z]	28.8 ns	40.3 a	48.4 d	74.1 b	67.4 cd
	CA 1	29.1 c	28.5	38.7 b	52.9 ab	74.0 b	76.1 ab
	CA 2	28.1 c	28.8	40.6 a	51.7 bc	73.6 b	71.2 bc
DT	Air	28.6 c	28.6	38.5 b	49.2 cd	73.2 b	63.8 d
	CA 1	40.4 b	27.4	26.8 c	55.3 a	81.3 a	78.3 a
	CA 2	43.0 a	27.8	26.3 c	54.5 ab	79.7 a	76.5 ab
LSD		*2.1162*	*1.4423*	*1.4800*	*3.1800*	*3.0500*	*6.6300*

[z] Means separation within columns using least significant differences (0.05)

Table 4

Total soluble solids (TSS) and titratable acidity (TA) of 'Angeleno' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmospheres (CA). Measurements were taken initially, after 18 days of storage, and after a further seven days of shelf life at 10°C.

	Atmosphere		After storage		After shelf life at 10°C	
			TSS (%)			
Initial		14.7				
7.5°C	Air		14.0	c[z]	13.9	b
	CA 1		15.0	a	14.3	ab
	CA 2		14.9	a	14.4	ab
DT	Air		14.8	ab	14.7	a
	CA 1		15.0	a	14.3	ab
	CA 2		14.3	bc	14.6	a
LSD			*0.5567*		*0.5940*	
			TA (% malic acid)			
Initial		0.74				
7.5°C	Air		0.82	a	0.83	ns
	CA 1		0.80	ab	0.77	
	CA 2		0.78	ab	0.79	
DT	Air		0.82	a	0.82	
	CA 1		0.74	b	0.82	
	CA 2		0.79	ab	0.81	
LSD			*0.0718*		*0.0799*	

[z] Means separation within columns using least significant differences (0.05)

Table 5

Disorders (aerated flesh and decay) of 'Angeleno' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C), in either air or controlled atmospheres (CA). Fruit were evaluated after 18 days of storage and after a further seven days of shelf life at 10°C.

	Atmosphere	After storage		After shelf life at 10°C	
		Aerated Flesh (%)			
7.5°C	Air	30.0	bc[z]	46.7	b
	CA 1	16.7	c	76.7	a
	CA 2	50.0	ab	76.7	a
DT	Air	30.0	bc	63.3	ab
	CA 1	43.3	ab	80.0	a
	CA 2	60.0	a	43.3	b
LSD		*24.419*		*26.773*	
		Decay (%)			
7.5°C	Air	0.0		16.7	ns
	CA 1	0.0		3.3	
	CA 2	0.0		23.3	
DT	Air	0.0		23.3	
	CA 1	0.0		10.0	
	CA 2	0.0		10.0	
LSD				*20.347*	

[z] Means separation within columns using least significant differences (0.05)

Table 6

Mass loss (%) and cumulative mass loss (%) of 'Angeleno' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmospheres (CA). Measurements were taken after 18 days of storage and after a further seven days of shelf life at 10°C.

	Atmosphere	After storage	During shelf life at 10°C	Cumulative mass loss (%)
		Mass loss (%)		
7.5°C	Air	0.41 b[z]	0.42 ns	0.83 ab
	CA 1	0.40 b	0.46	0.86 ab
	CA 2	0.60 a	0.45	1.05 a
DT	Air	0.31 bc	0.52	0.83 ab
	CA 1	0.19 c	0.37	0.56 b
	CA 2	0.31 bc	0.44	0.75 ab
LSD		*0.1595*	*0.2965*	*0.3547*

[z] Means separation within columns using least significant differences (0.05)

Table 7

Total soluble solids (TSS) and titratable acidity (TA) of 'Songold' plum fruit stored at -0.5°C for six days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 19 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day six, after 24 days storage, after 43 days storage and after a shelf life period on day 49.

	Atmosphere	6 Days	24 Days	43 Days	49 Days
			TSS (%)		
Initial		13.4			
Long DT	Air		13.7 ns[z]	13.5 ab	13.4 b
	CA		13.4	13.8 a	14.1 a
Short DT	Air		13.7	13.3 b	13.7 ab
	CA		13.7	13.4 ab	13.4 b
LSD			*0.7095*	*0.4142*	*0.4266*
			TA (% malic acid)		
Initial		1.46			
Long DT	Air		1.22 b	1.14 b	1.03 ns
	CA		1.29 ab	1.23 a	1.11
Short DT	Air		1.29 ab	1.21 ab	1.07
	CA		1.34 a	1.26 a	1.07
LSD			*0.0780*	*0.0790*	*0.1510*

[z] Means separation within columns using least significant differences (0.05)

Table 8

Disorders (internal browning, gel breakdown and decay) of 'Songold' plum fruit stored at -0.5°C for six days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 19 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken after the shelf life period on day 49.

	Atmosphere	Internal browning (%)	Gel breakdown (%)	Decay (%)
Long DT	Air	0.0 ns[z]	44.4 a	2.5 ns
	CA	2.8	13.9 b	2.5
Short DT	Air	3.1	17.0 b	5.0
	CA	0.0	11.1 b	0.0
LSD		*6.4388*	*13.642*	*7.0321*

[z] Means separation within columns using least significant differences (0.05)

Table 9

Mass loss (%) and cumulative mass (%) loss of 'Songold' plum fruit stored at -0.5°C for six days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 19 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially after six days, after 24 days storage, after 43 days storage and after a shelf life period on day 49.

	Atmosphere	24 Days	43 Days	49 Days	Cumulative mass loss (%)
		Mass loss (%)			
Long DT	Air	0.10 a[z]	0.32 ns	0.41 a	0.83 a
	CA	0.06 ab	0.24	0.32 a	0.61 ab
Short DT	Air	0.05 ab	0.39	0.40 a	0.84 a
	CA	0.01 b	0.32	0.09 b	0.41 b
LSD		*0.0647*	*0.1880*	*0.1890*	*0.2796*

[z] Means separation within columns using least significant differences (0.05)

Table 10

Colour (chroma, lightness and hue angle) of 'Angeleno' plum fruit flesh stored at -0.5°C for seven days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 18 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day seven, after 25 days storage, after 43 days storage and after a shelf life period on day 49.

	Atmosphere	7 Days	25 Days	43 Days	49 Days
			Chroma		
Initial		33.7			
Long DT	Air		32.6 a[z]	31.9 c	26.9 c
	CA		31.6 ab	40.1 a	29.4 ab
Short DT	Air		31.2 b	35.3 b	28.0 bc
	CA		32.0 ab	38.8 a	30.7 a
LSD			*1.3155*	*1.8950*	*2.0483*
			Lightness		
Initial		64.3			
Long DT	Air		54.4 ns	59.4 c	46.6 c
	CA		54.8	63.0 a	55.9 a
Short DT	Air		55.9	61.1 b	54.0 b
	CA		55.5	62.6 a	55.8 a
LSD			*1.7542*	*1.0910*	*1.6441*
			Hue (°)		
Initial		92.4			
Long DT	Air		82.4 b	90.3 b	65.3 c
	CA		87.3 a	94.5 a	87.8 a
Short DT	Air		89.0 a	91.0 b	79.4 b
	CA		86.7 a	96.1 a	85.6 a
LSD			*2.5228*	*2.7819*	*3.3724*

[z] Means separation within columns using least significant differences (0.05)

Table 11

Total soluble solids (TSS) and titratable acidity (TA) of 'Angeleno' plum fruit stored at -0.5°C for seven days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 18 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day seven, after 25 days storage, after 43 days storage and after a shelf life period on day 49.

	Atmosphere	7 Days	25 Days	43 Days	49 Days
			TSS (%)		
Initial		12.7			
Long DT	Air		12.2 ns[z]	12.4 ns	12.6 ns
	CA		12.3	12.6	12.6
Short DT	Air		12.3	12.4	12.7
	CA		12.4	12.2	12.7
LSD			*0.5590*	*0.5537*	*0.4743*
			TA (% malic acid)		
Initial		1.36			
Long DT	Air		1.20 ab	1.11 ns	1.04 b
	CA		1.25 ab	1.06	1.20 a
Short DT	Air		1.17 b	1.09	1.04 b
	CA		1.30 a	1.07	1.24 a
LSD			*0.1142*	*0.0574*	*0.0949*

[z] Means separation within columns using least significant differences (0.05)

Table 12

Disorders (internal browning, aerated flesh and decay) of 'Angeleno' plum fruit stored at -0.5°C for seven days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 18 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken after the shelf life period on day 49.

	Atmosphere	Internal browning (%)	Aerated flesh (%)	Decay (%)
Long DT	Air	11.1 ab[z]	5.6 b	0.0 ns
	CA	17.0 a	11.1 b	2.8
Short DT	Air	0.0 b	19.4 ab	0.0
	CA	0.0 b	25.7 ab	0.0
LSD		*12.954*	*14.326*	*4.2735*

[z] Means separation within columns using least significant differences (0.05)

Table 13

Mass loss (%) and cumulative mass loss (%) of 'Angeleno' plum fruit stored at -0.5°C for seven days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 18 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day 7, after 25 days storage, after 43 days storage and after a shelf life period on day 49.

	Atmosphere	25 Days	43 Days	49 Days	Cumulative mass loss (%)
		Mass loss (%)			
Long DT	Air	0.36 ns[z]	0.26 a	0.28 ns	0.89 a
	CA	0.23	0.12 ab	0.14	0.49 b
Short DT	Air	0.37	0.04 b	0.26	0.67 ab
	CA	0.28	0.16 ab	0.10	0.54 b
LSD		*0.1505*	*0.1729*	*0.2435*	*0.2902*

[z] Means separation within columns using least significant differences (0.05)

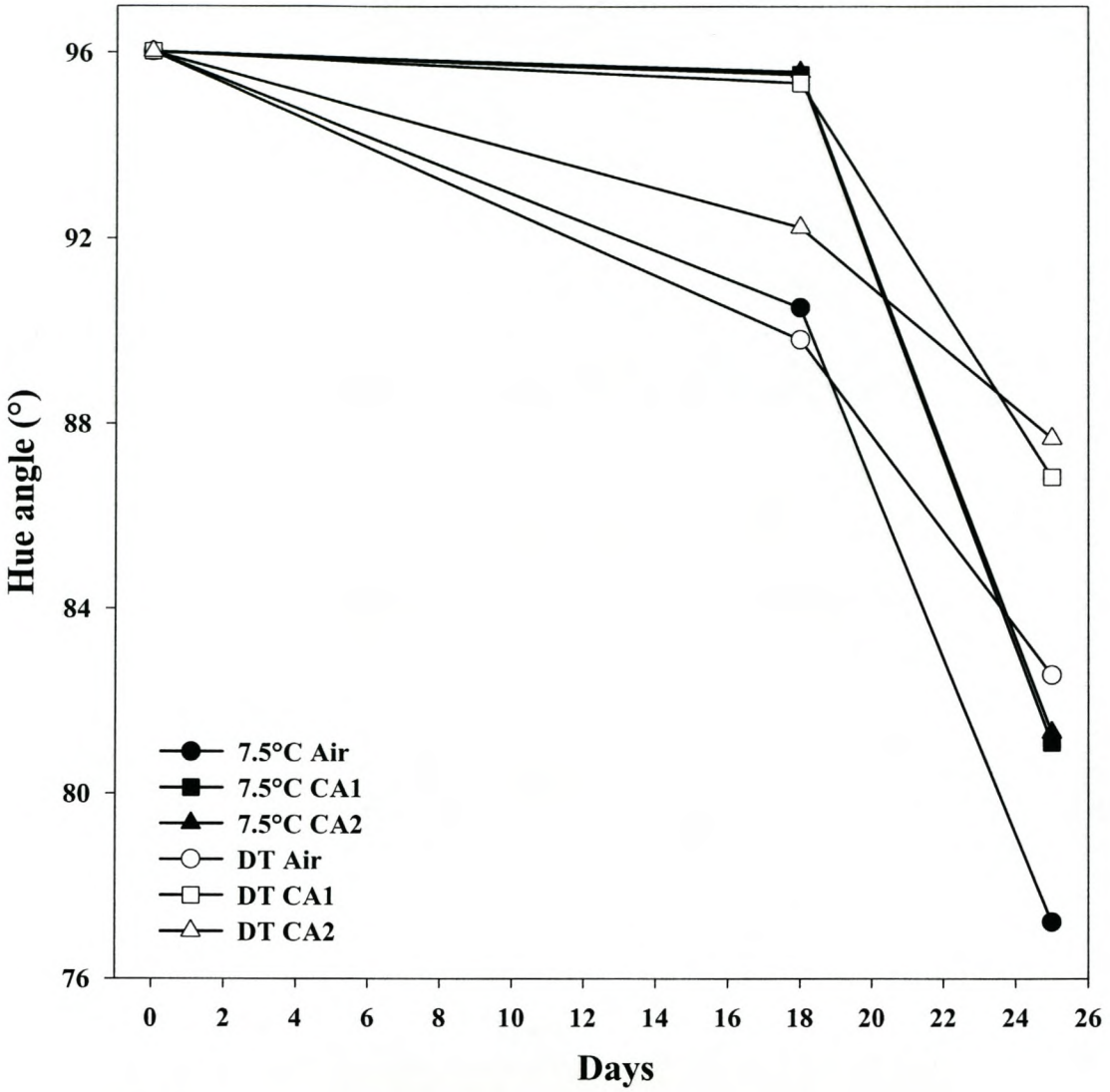

Fig. 1. Hue angle (°) of 'Songold' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmospheres (CA). Measurements were taken initially, after 18 days of storage (*LSD = 1.0385*), and after a further seven days of shelf life at 10°C in air (*LSD = 3.4928*).

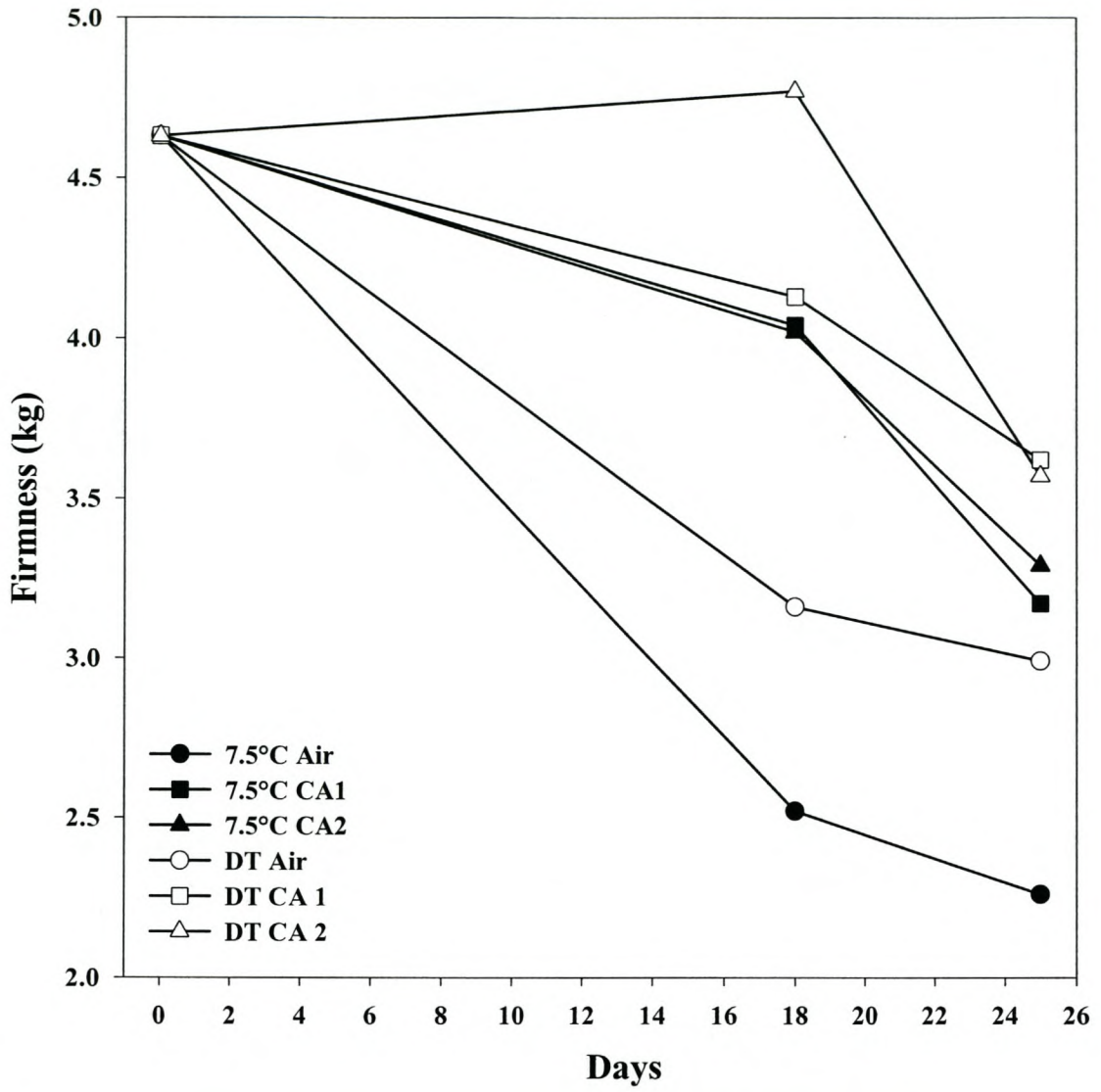

Fig. 2. Firmness (kg) of 'Songold' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmospheres (CA). Measurements were taken initially, after 18 days of storage (*LSD = 0.3973*), and after a further seven days of shelf life at 10°C in air (*LSD = 0.3678*).

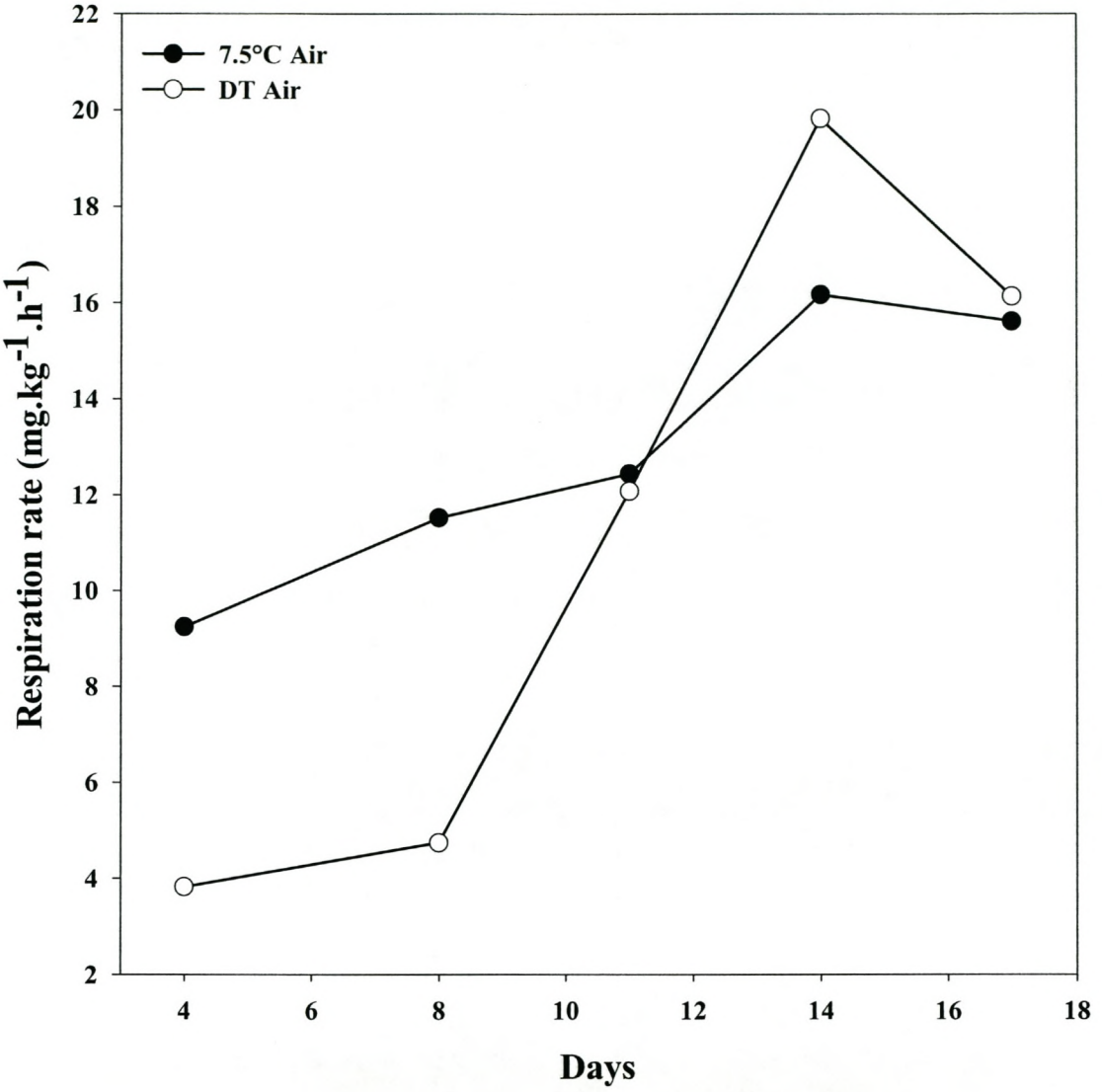

Fig. 3. Respiration rate (CO_2 evolved in $mg.kg^{-1}.h^{-1}$) of 'Songold' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in air (*LSD = 1.6706*).

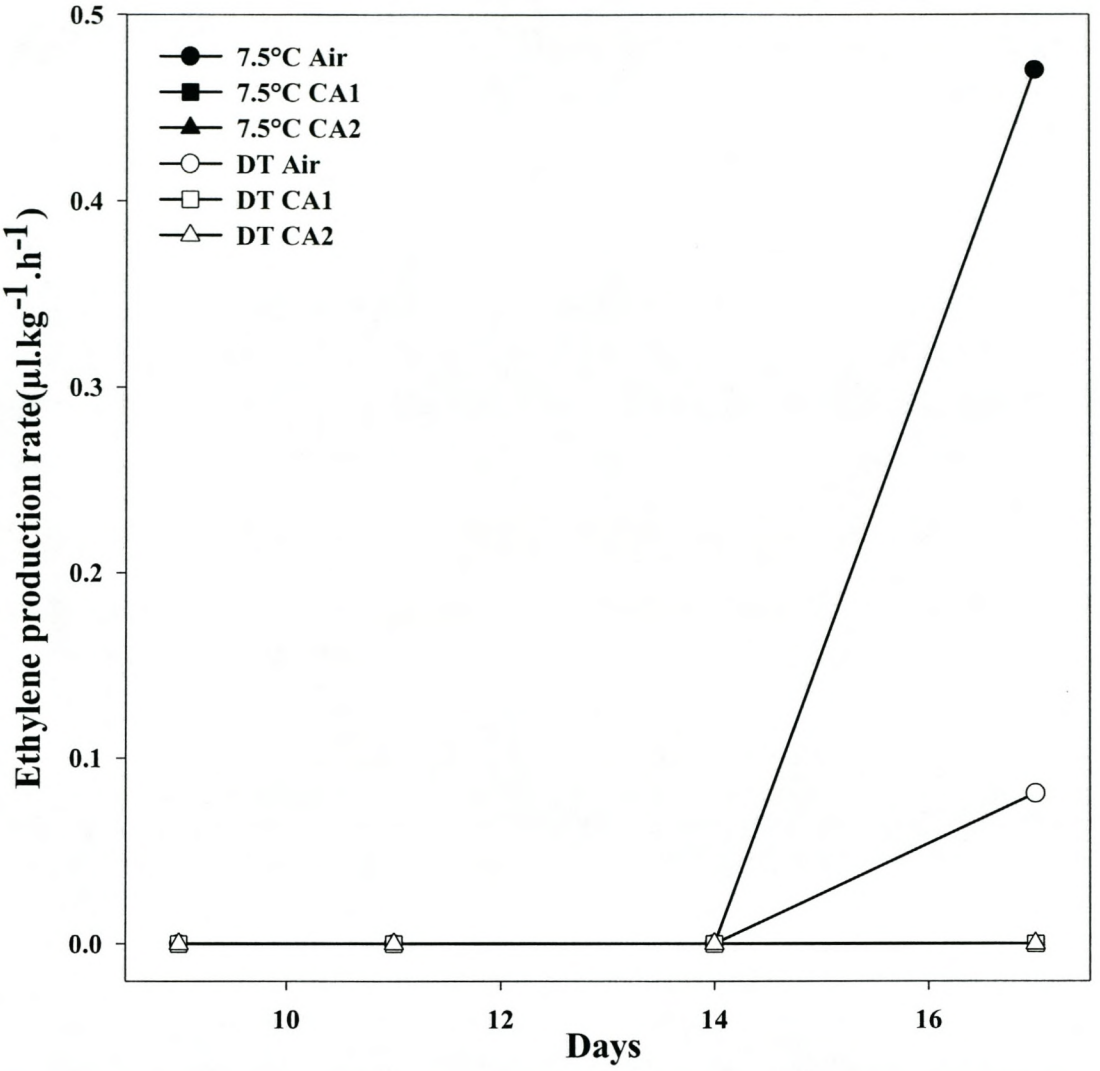

Fig. 4. Ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of 'Songold' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmosphere (CA) (*LSD = 0.0756*).

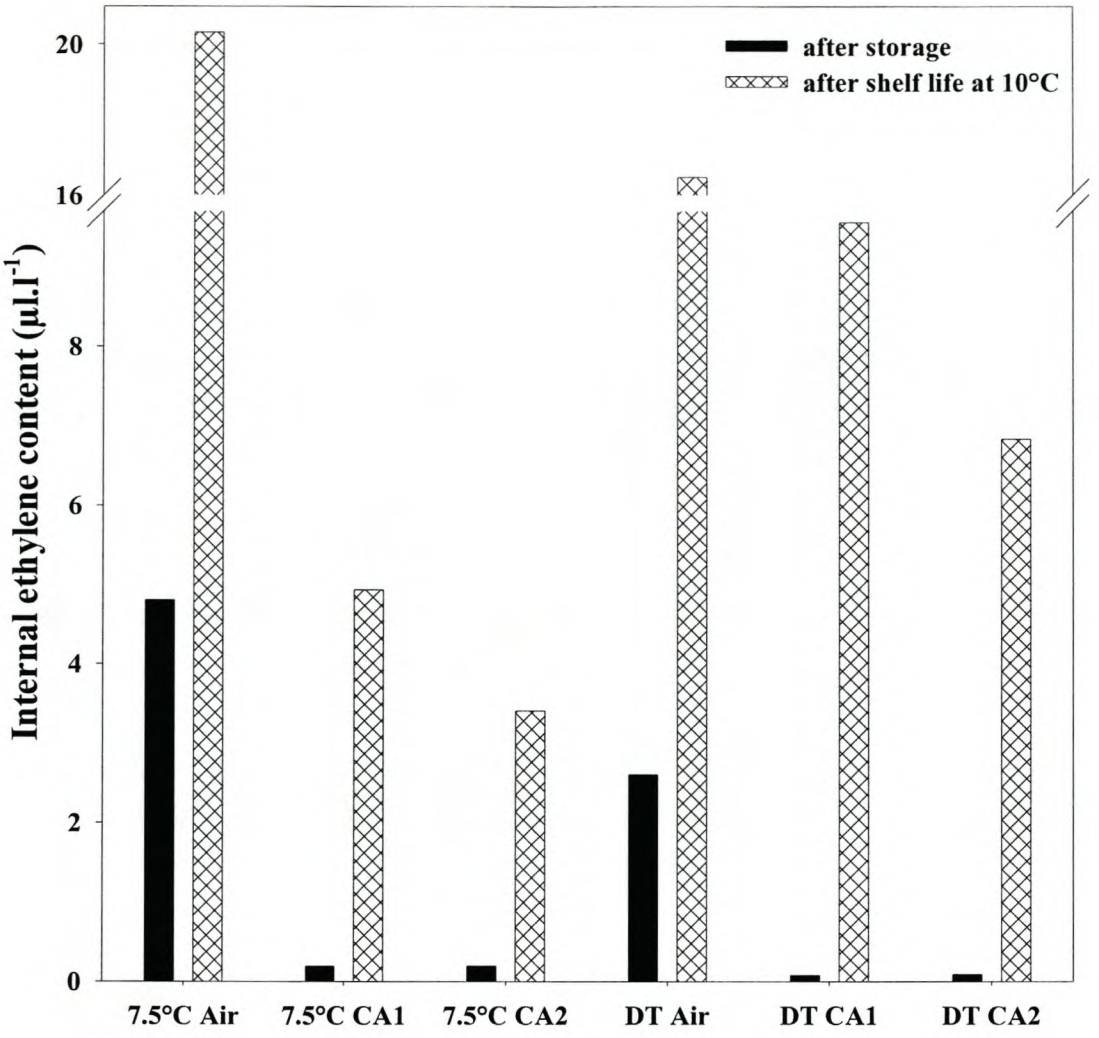

Fig. 5. Internal ethylene content ($\mu l.l^{-1}$) of 'Songold' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmospheres (CA). Measurements were taken after 18 days of storage (*LSD = 1.1842*) and after a further seven days of shelf life at 10°C in air (*LSD = 8.6830*).

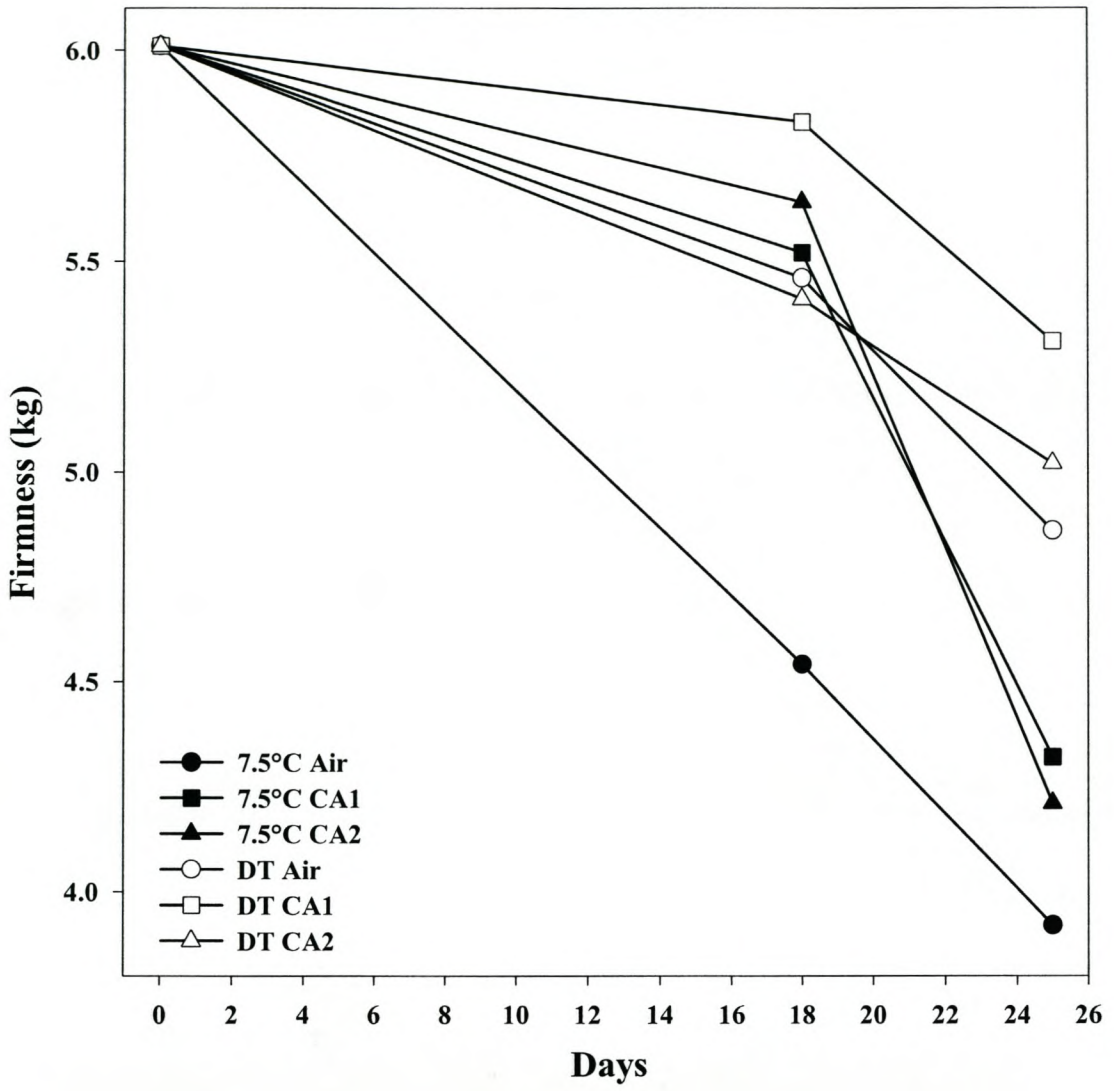

Fig. 6. Firmness (kg) of 'Angeleno' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmospheres (CA). Measurements were taken initially, after 18 days of storage (*LSD = 0.5615*), and after a further seven days of shelf life at 10°C in air (*LSD = 0.6060*).

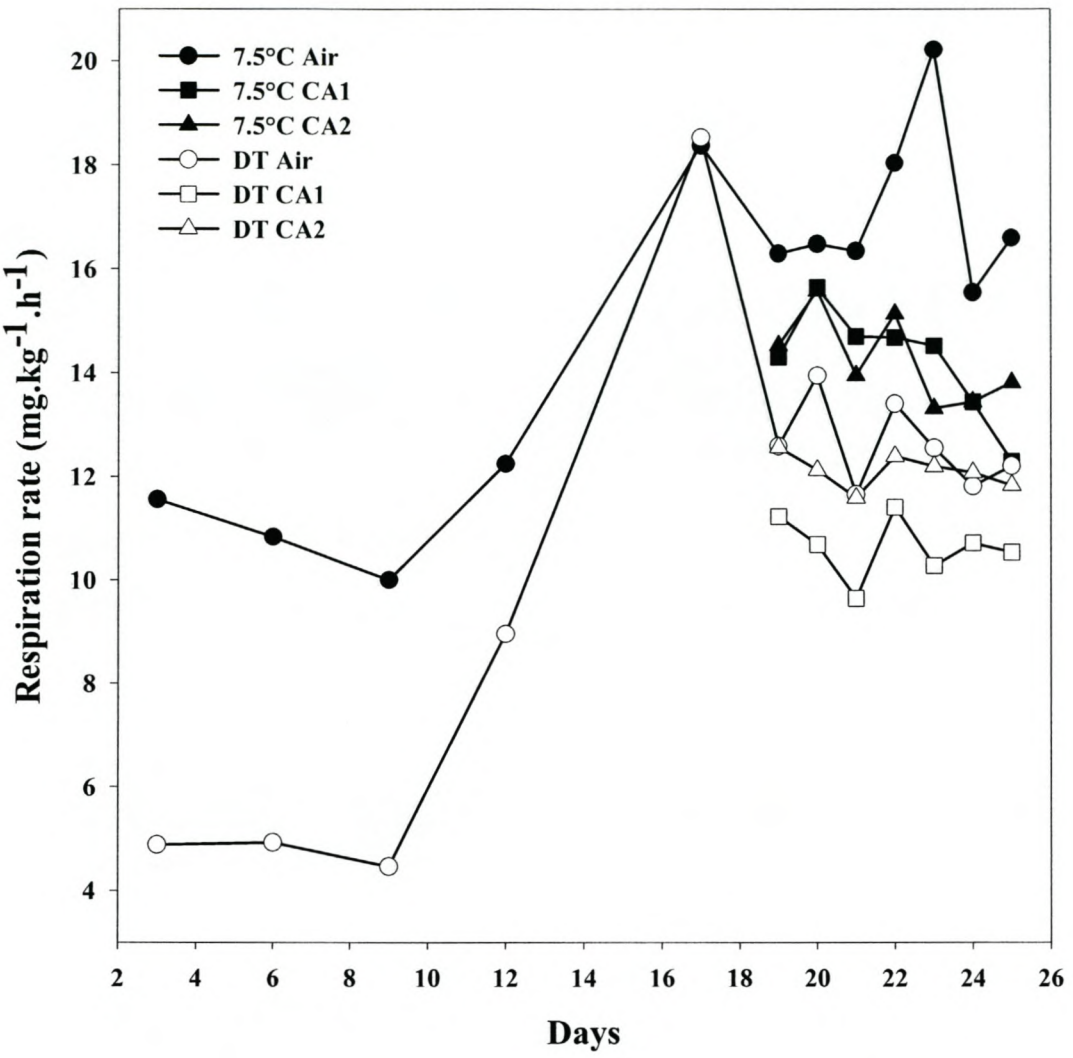

Fig. 7. Respiration rate (CO_2 evolved in $mg.kg^{-1}.h^{-1}$) of 'Angeleno' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmosphere (CA). After 18 days storage (*LSD = 2.560*) the fruit were stored at 10°C for seven days simulating a shelf life period in air (*LSD = 2.7027*).

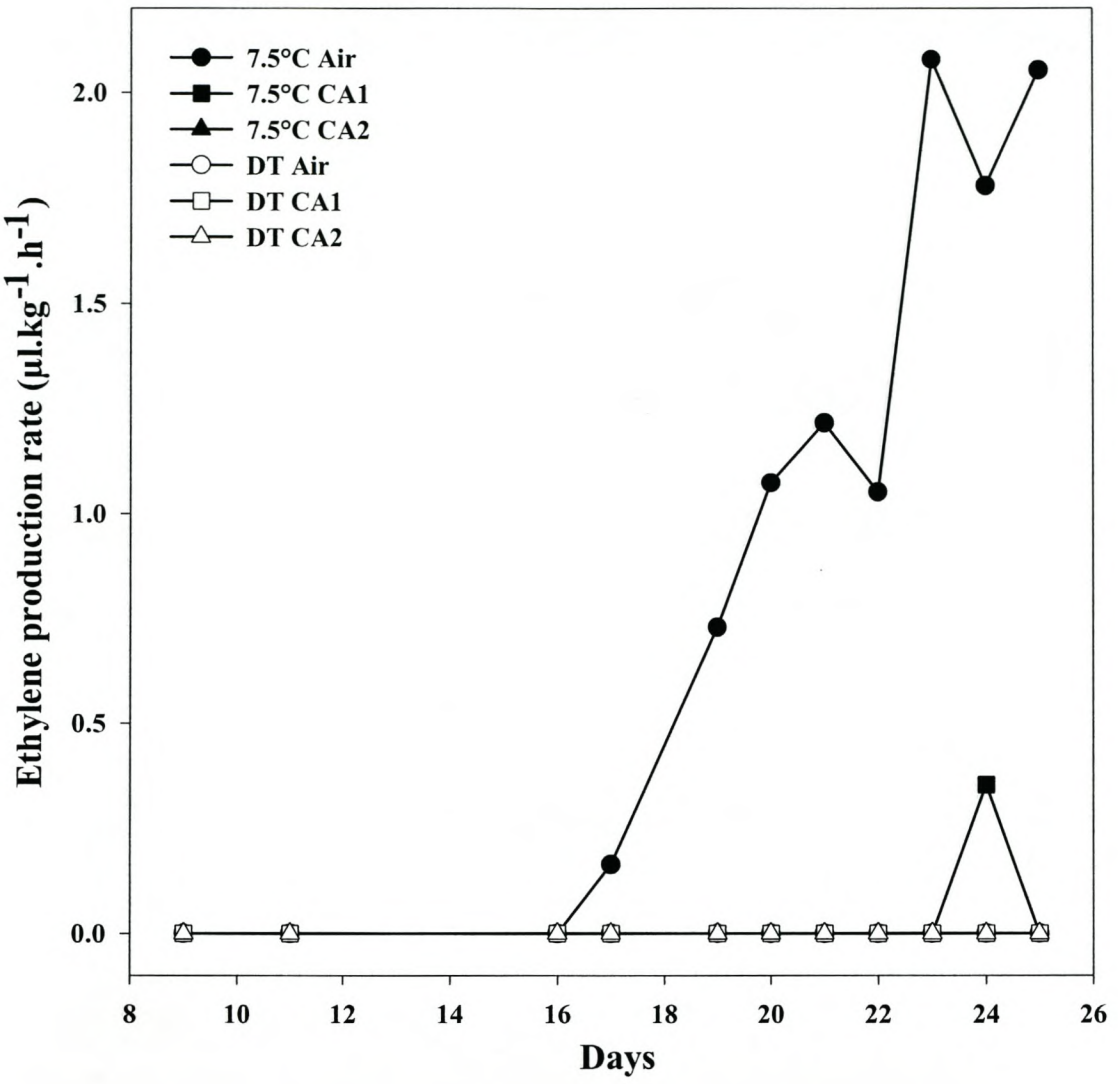

Fig. 8. Ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of 'Angeleno' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmosphere (CA). After 18 days storage (*LSD = 0.1656*) the fruit were stored at 10°C for seven days simulating a shelf life period in air (*LSD = 0.4063*).

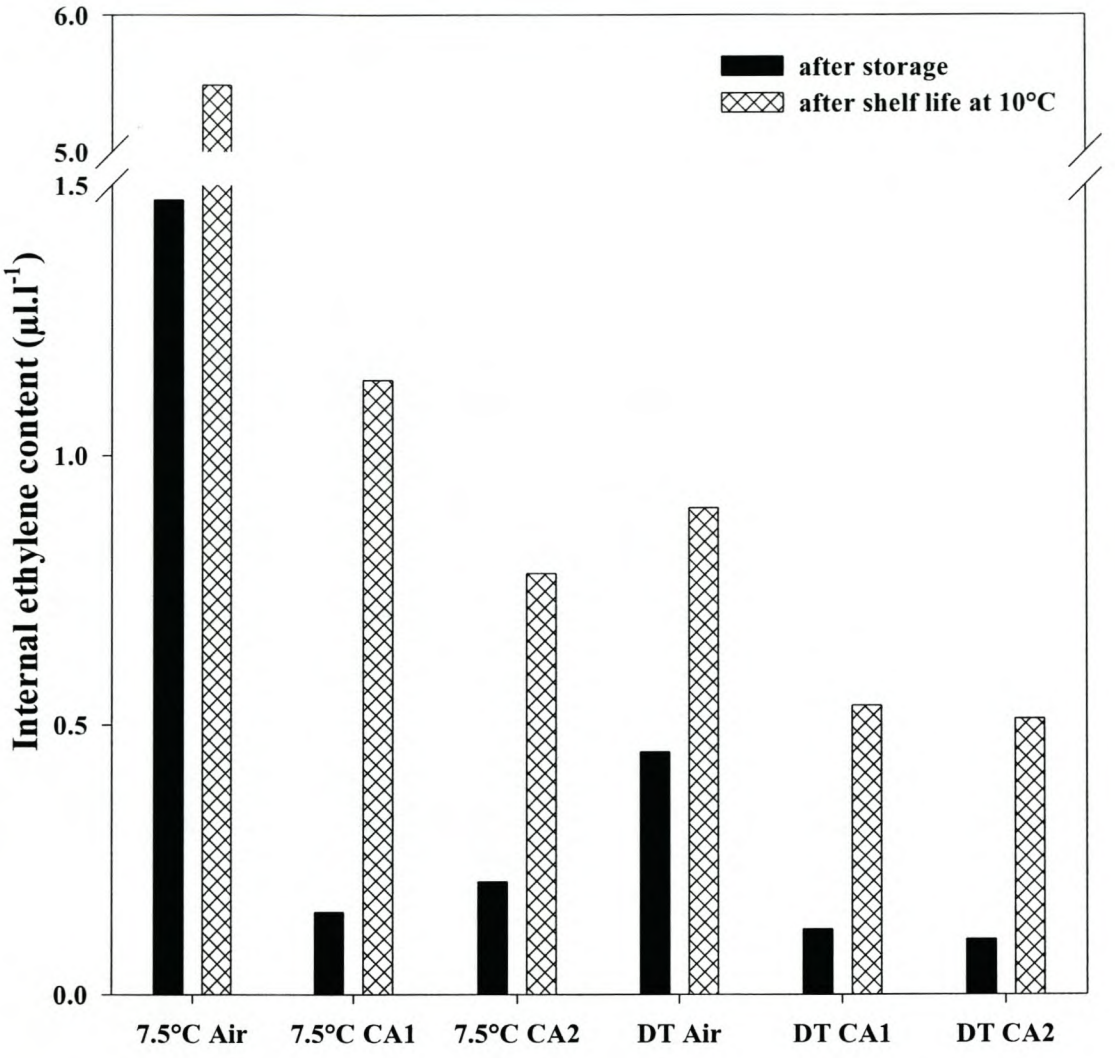

Fig. 9. Internal ethylene content ($\mu l.l^{-1}$) of 'Angeleno' plum fruit stored for 18 days at 7.5°C or under a dual temperature regime (10 days at -0.5°C and eight days at 7.5°C) (DT), in either air or controlled atmospheres (CA). Measurements were taken after 18 days of storage (*LSD = 0.1977*) and after a further seven days of shelf life at 10°C in air (*LSD = 1.0380*).

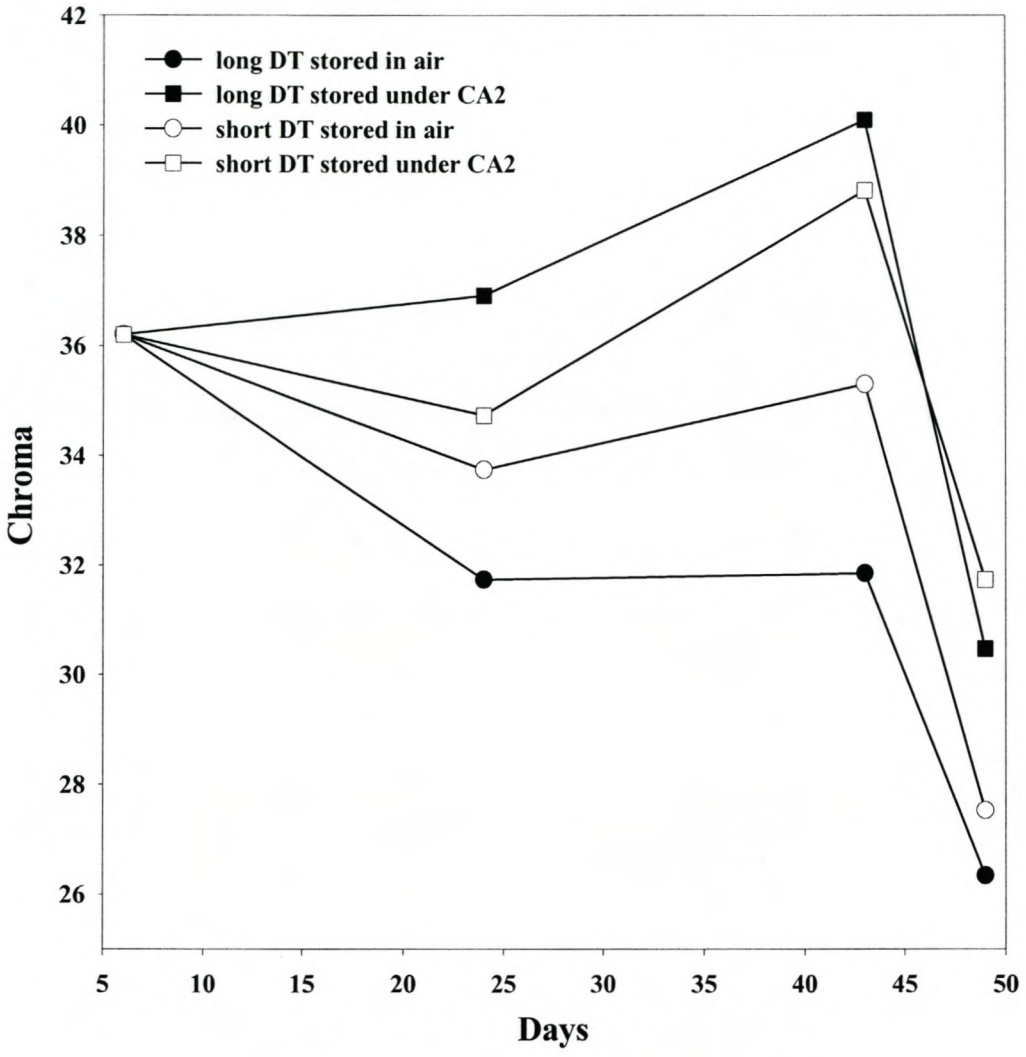

Fig. 10. Chroma of 'Songold' plum fruit stored at -0.5°C for six days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 19 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day six, after 24 days storage (*LSD = 2.6873*), after 43 days storage (*LSD = 1.8950*), and after a shelf life period on day 49 (*LSD = 2.1341*).

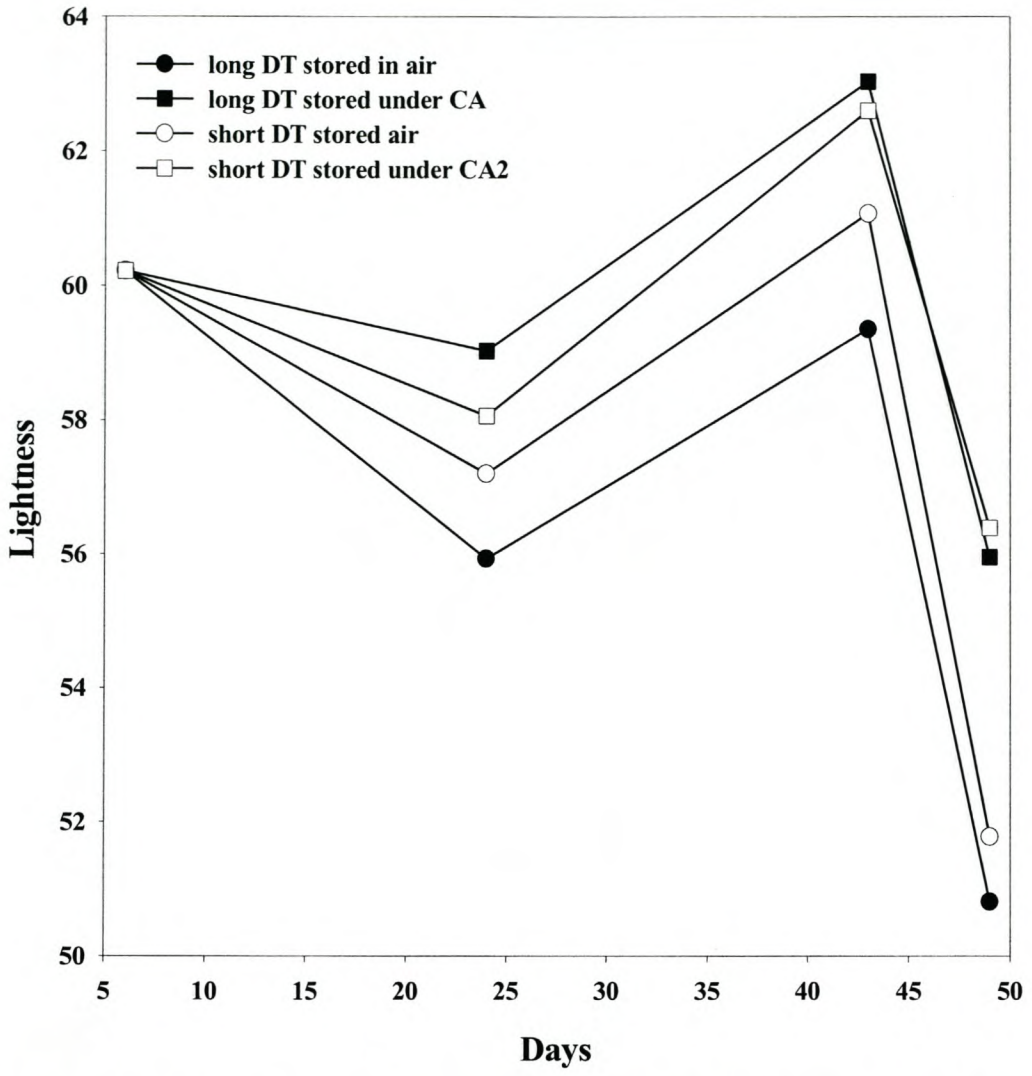

Fig. 11. Lightness of 'Songold' plum fruit stored at -0.5°C for six days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 19 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day six, after 24 days storage (*LSD = 1.5059*), after 43 days storage (*LSD = 1.0910*), and after a shelf life period on day 49 (*LSD = 1.1758*).

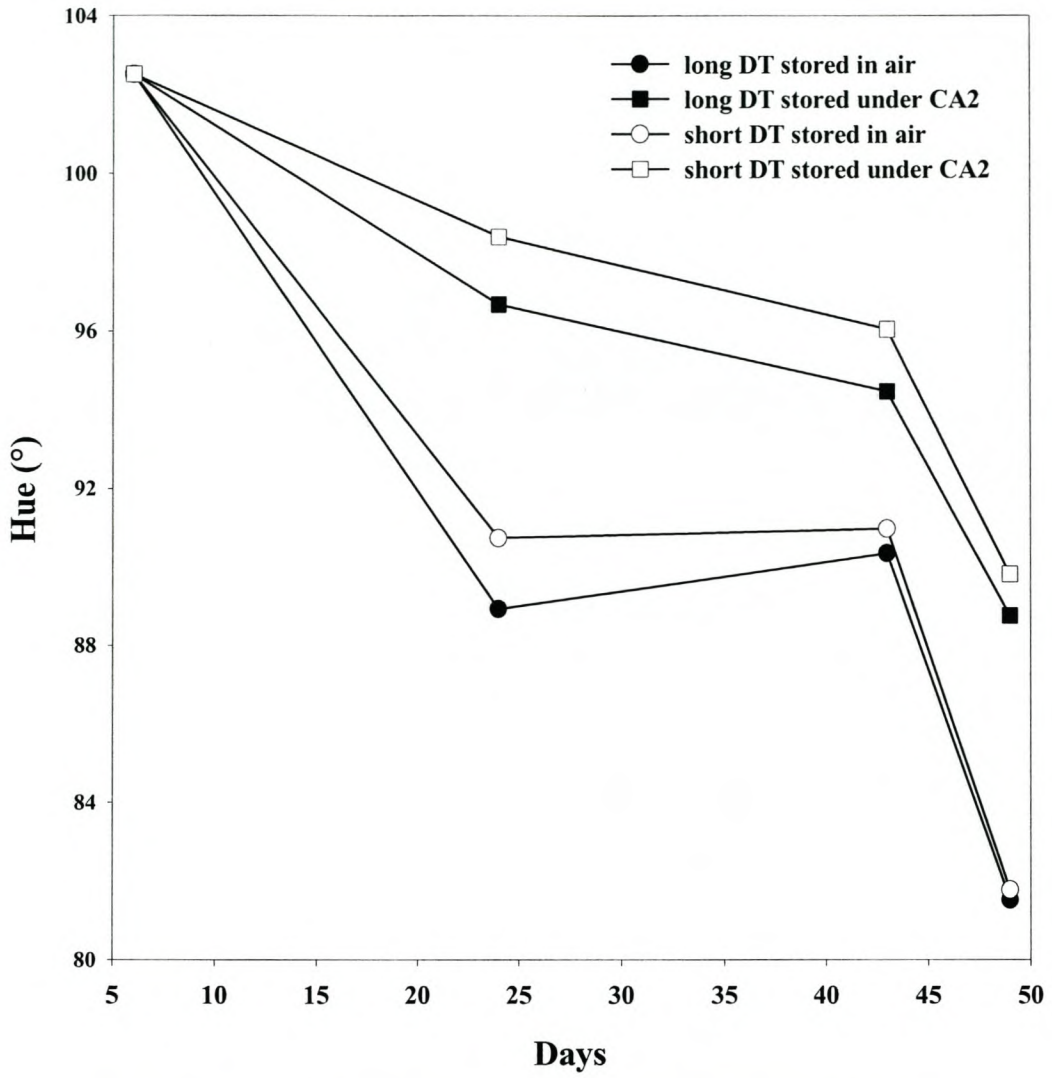

Fig. 12. Hue angle (°) of 'Songold' plum fruit stored at -0.5°C for six days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 19 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day six, after 24 days storage (*LSD = 3.2817*), after 43 days storage (*LSD = 2.7819*) and after a shelf life period on day 49 (*LSD = 4.3924*).

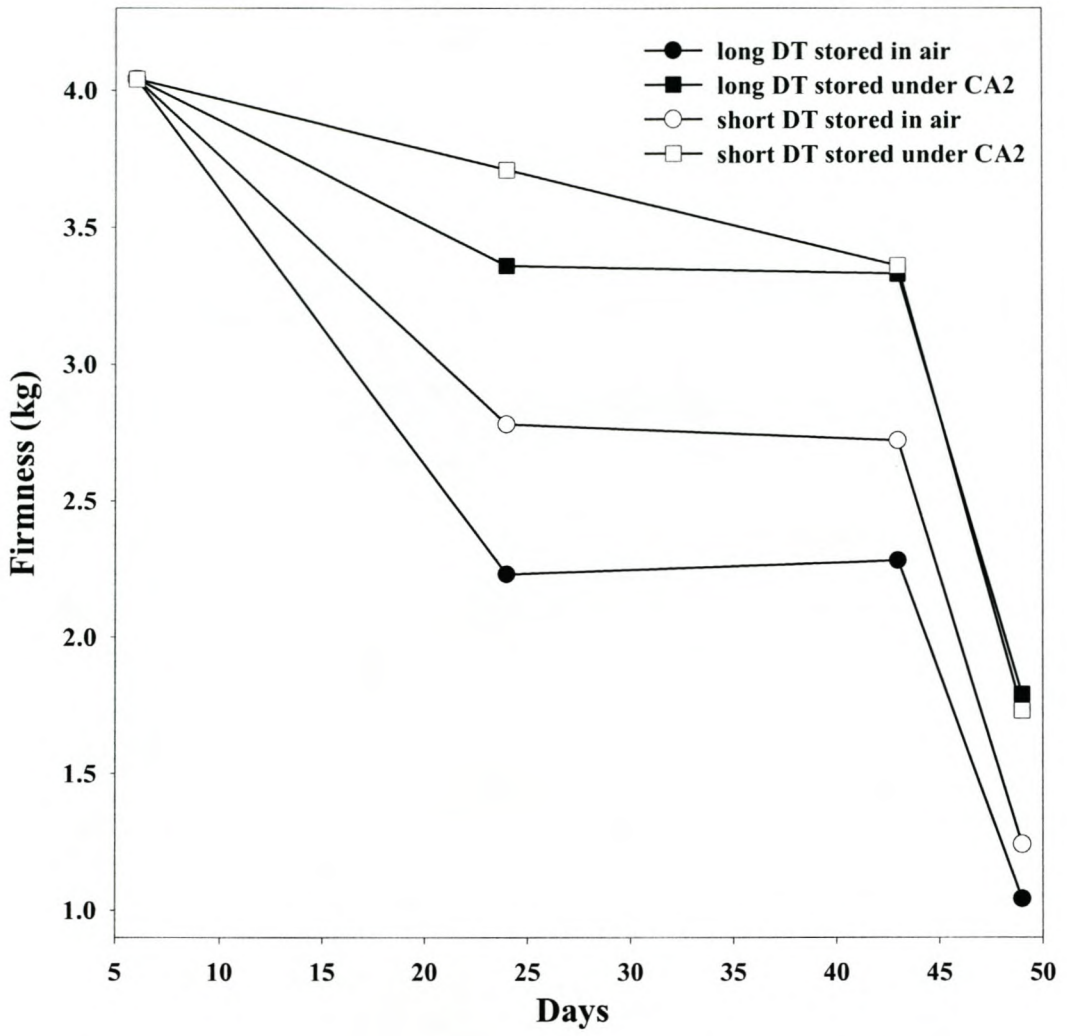

Fig. 13. Firmness (kg) of 'Songold' plum fruit stored at -0.5°C for six days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 19 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day six, after 24 days storage (*LSD = 0.5105*), after 43 days storage (*LSD = 0.3497*) and after a shelf life period on day 49 (*LSD = 0.1545*).

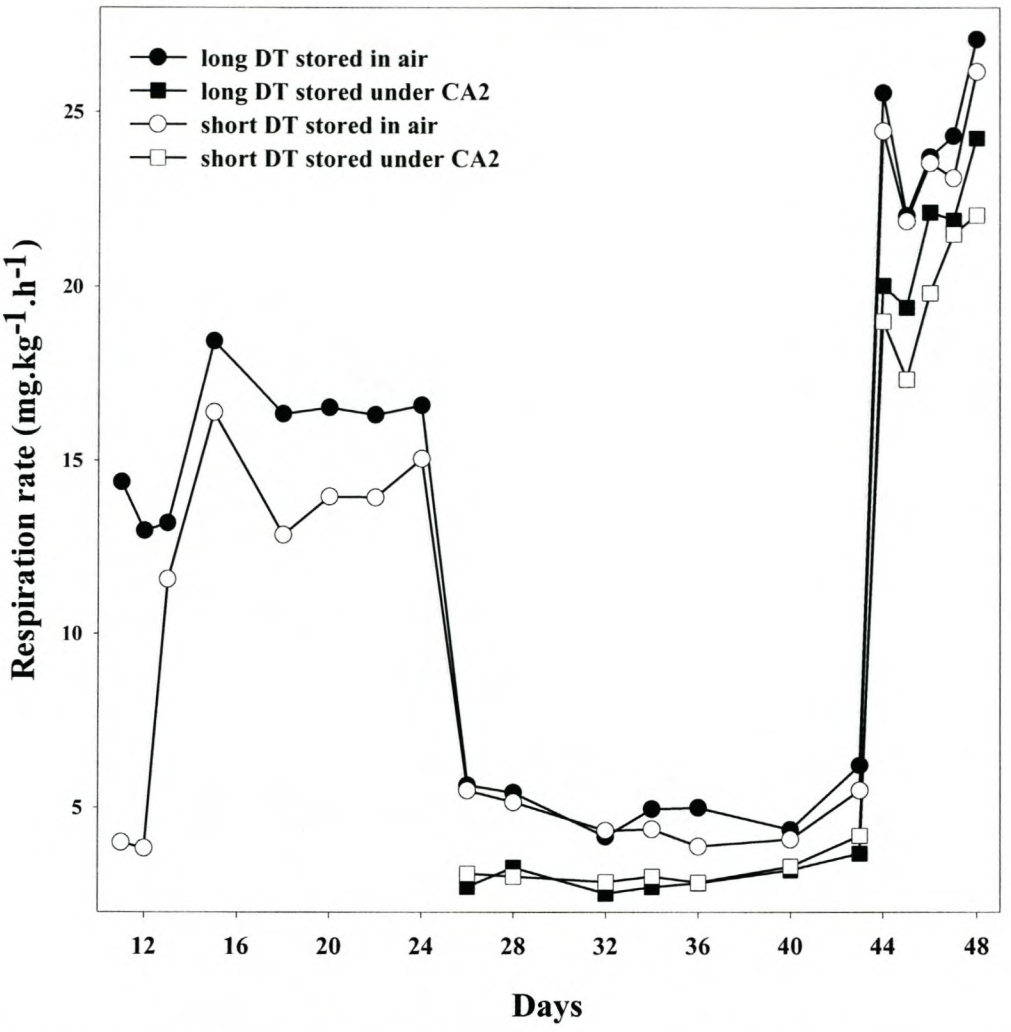

Fig. 14. Respiration rate (CO_2 evolved in $mg.kg^{-1}.h^{-1}$) of 'Songold' plum fruit stored at -0.5°C for six days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 19 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. LSD values were taken for the period up to 24 days storage (*LSD = 1.4451*) and after a shelf life period on day 49 (*LSD = 3.8714*).

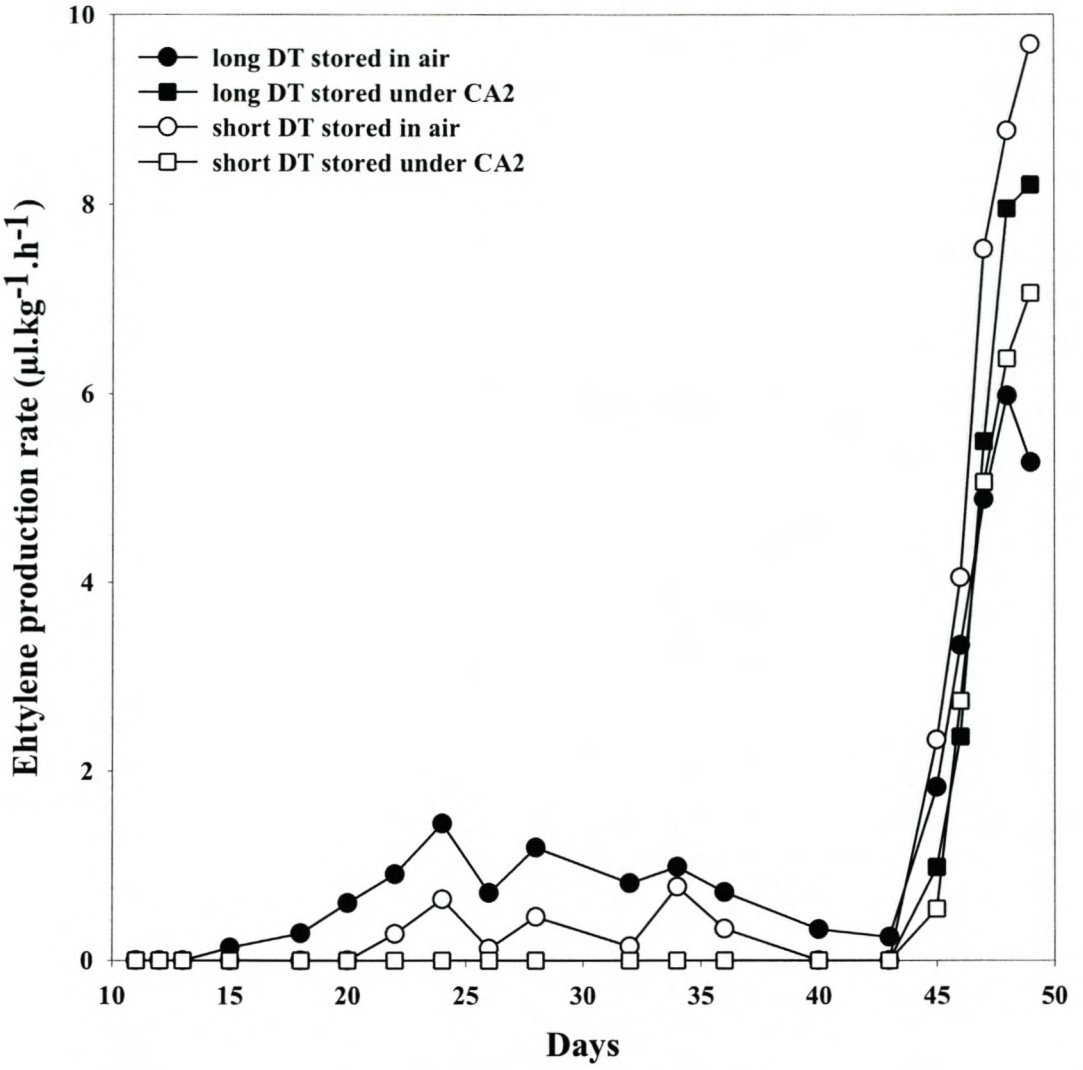

Fig. 15. Ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of 'Songold' plum fruit stored at -0.5°C for six days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 19 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days (*LSD = 0.804*).

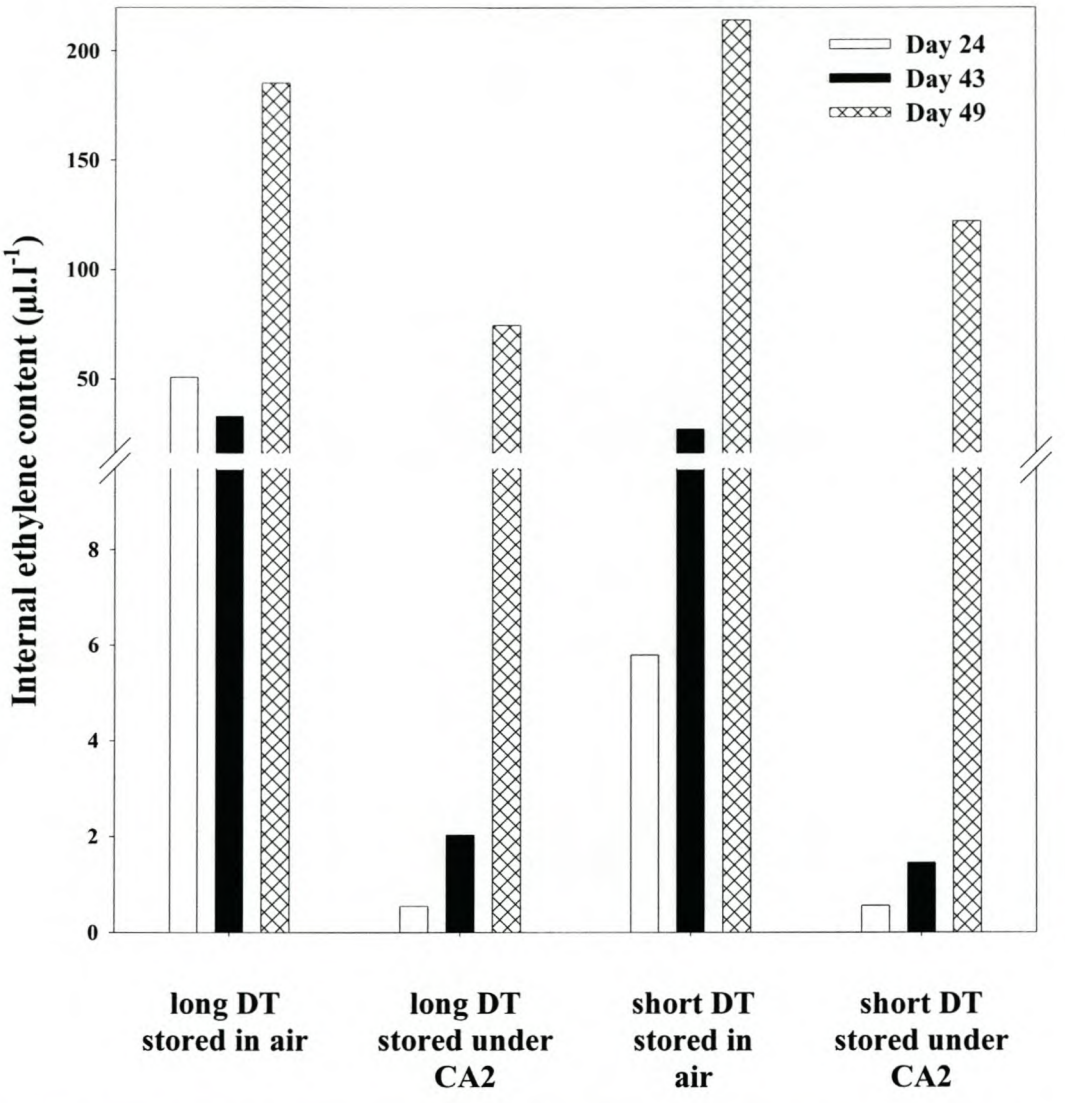

Fig. 16. Internal ethylene content ($\mu l.l^{-1}$) of 'Songold' plum fruit stored at -0.5°C for six days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 19 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day six, after 24 days storage (*LSD = 13.494*), after 43 days storage (*LSD = 3.8563*) and after a shelf life period on day 49 (*LSD = 36.574*).

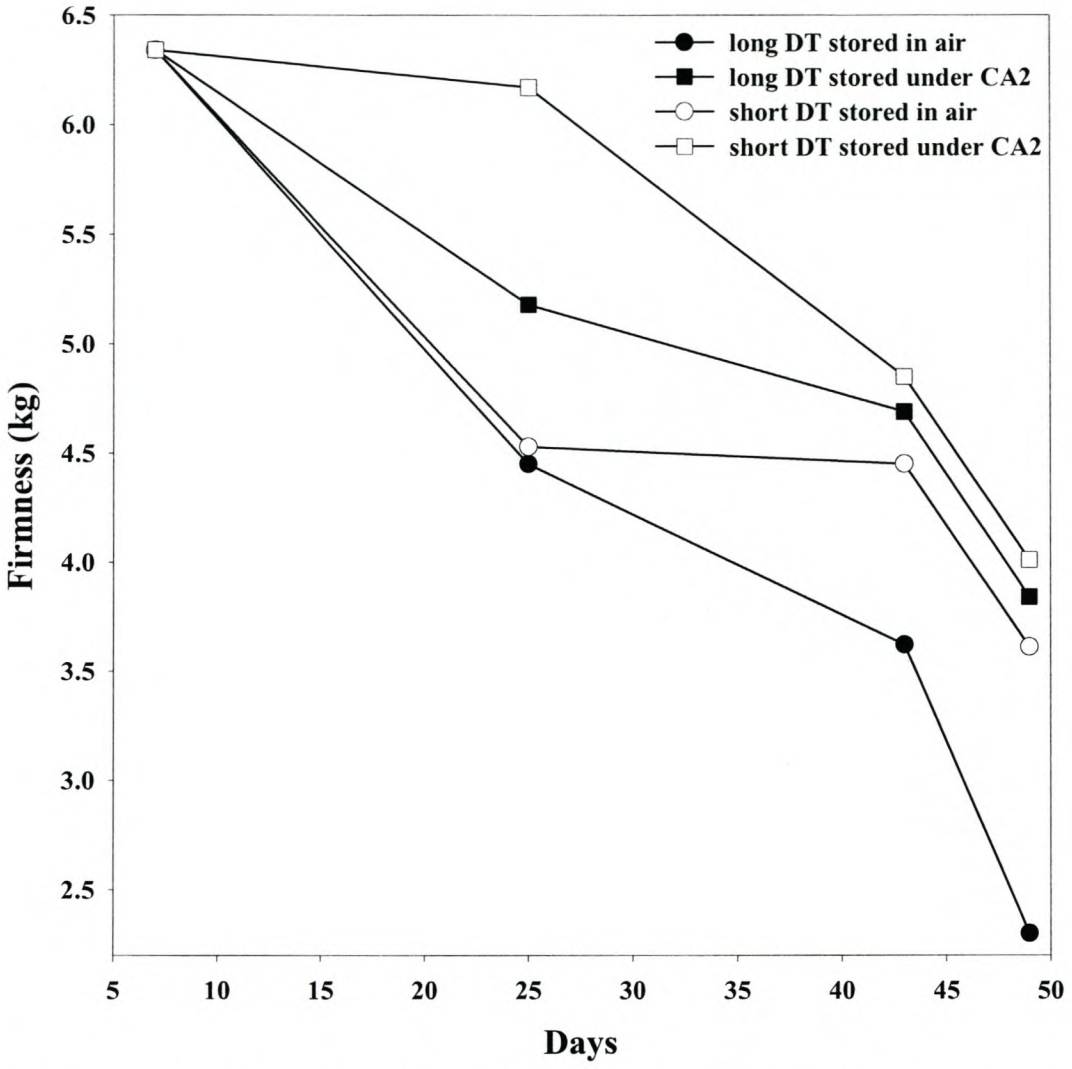

Fig. 17. Firmness (kg) of 'Angeleno' plum fruit stored at -0.5°C for seven days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 18 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day seven, after 25 days storage (*LSD = 0.5529*), after 43 days storage (*LSD = 0.6833*) and after a shelf life period on day 49 (*LSD = 0.3355*).

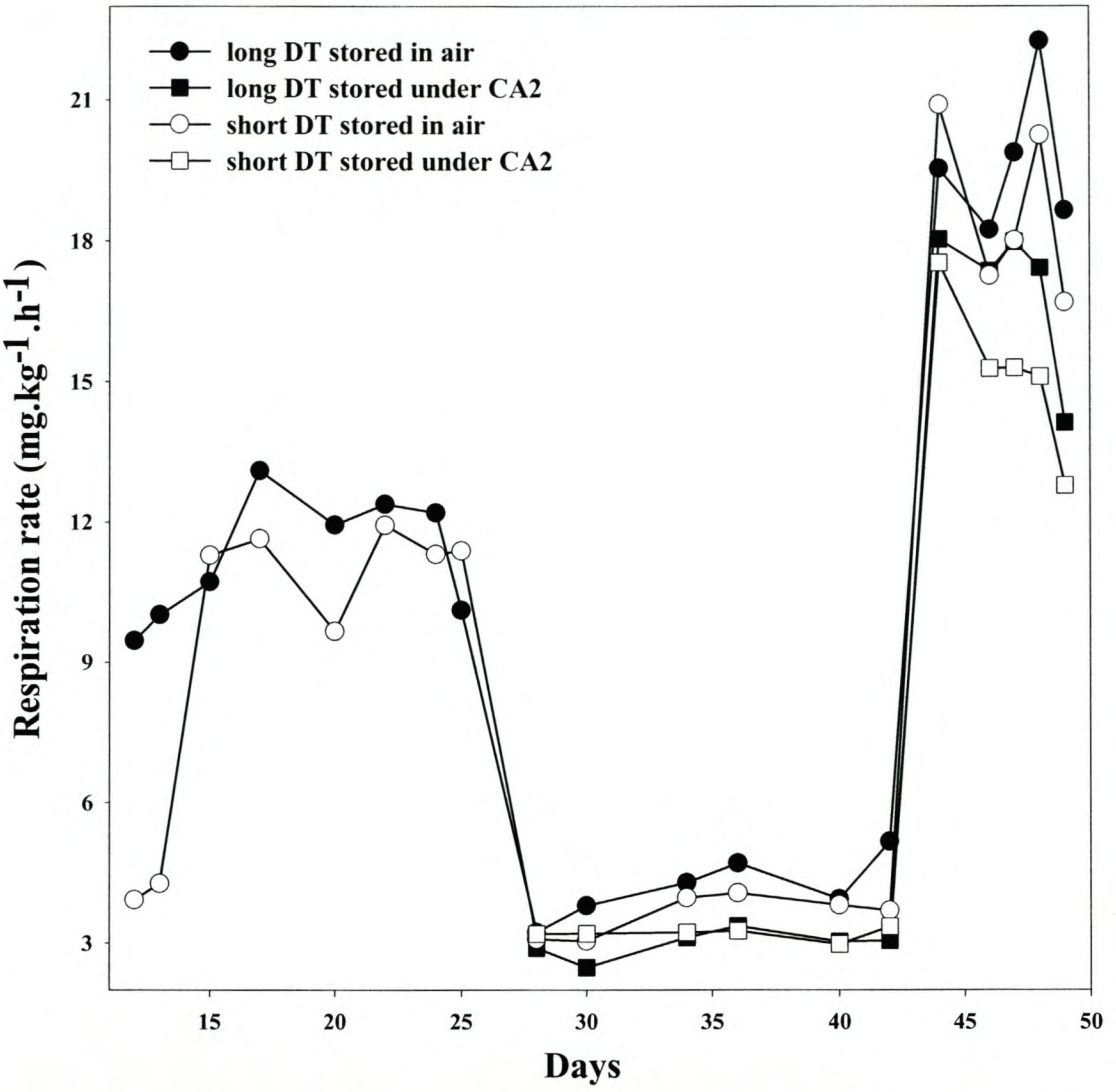

Fig. 18. Respiration rate (CO_2 evolved in $mg.kg^{-1}.h^{-1}$) of 'Angeleno' plum fruit stored at -0.5°C for seven days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 18 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. LSD values were taken for the period up to 25 days storage (*LSD = 1.6189*) and after a shelf life period on day 49 (*LSD = 1.3701*).

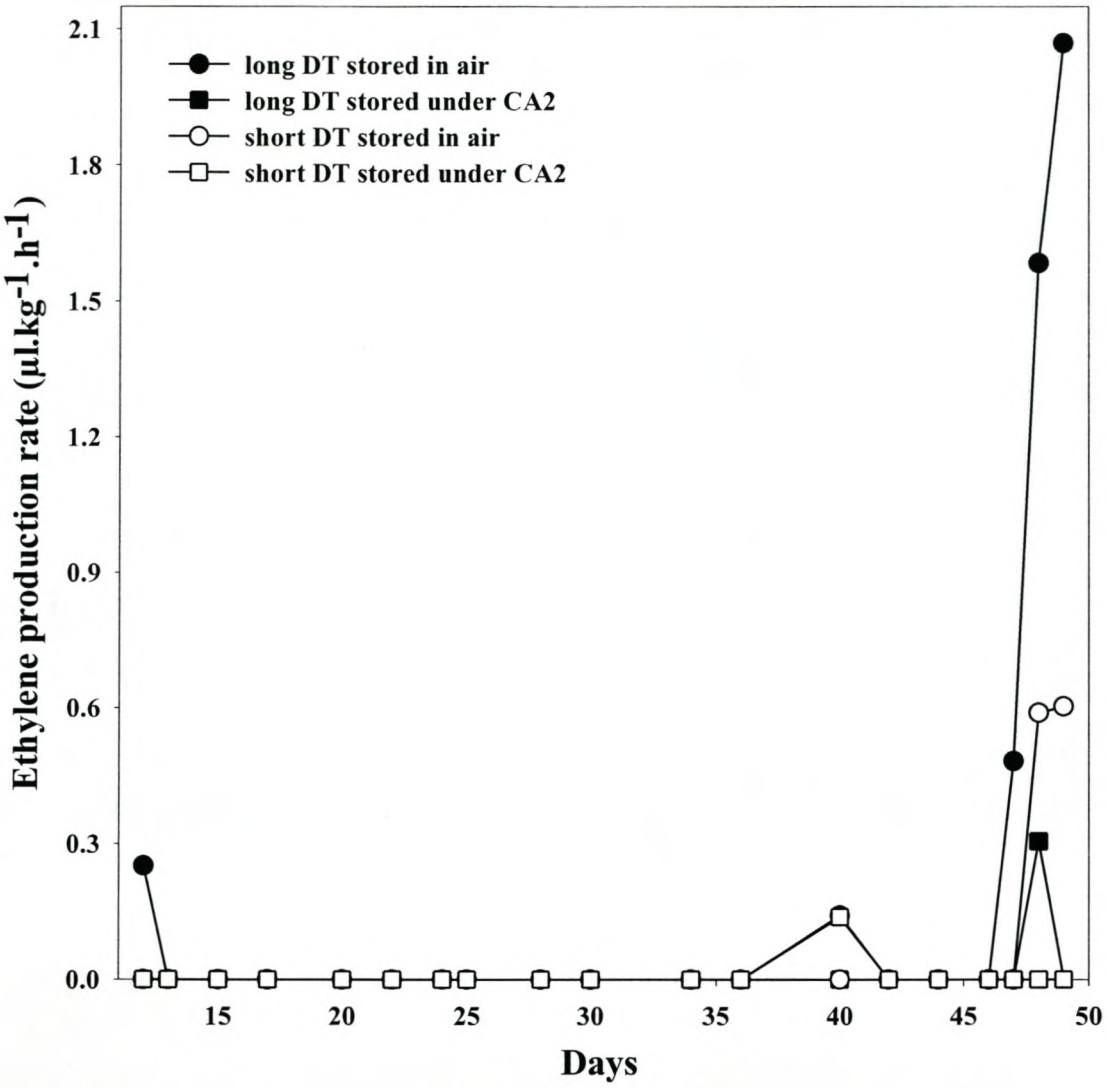

Fig. 19. Ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of 'Angeleno' plum fruit stored at -0.5°C for seven days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 18 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days (*LSD = 0.2975*).

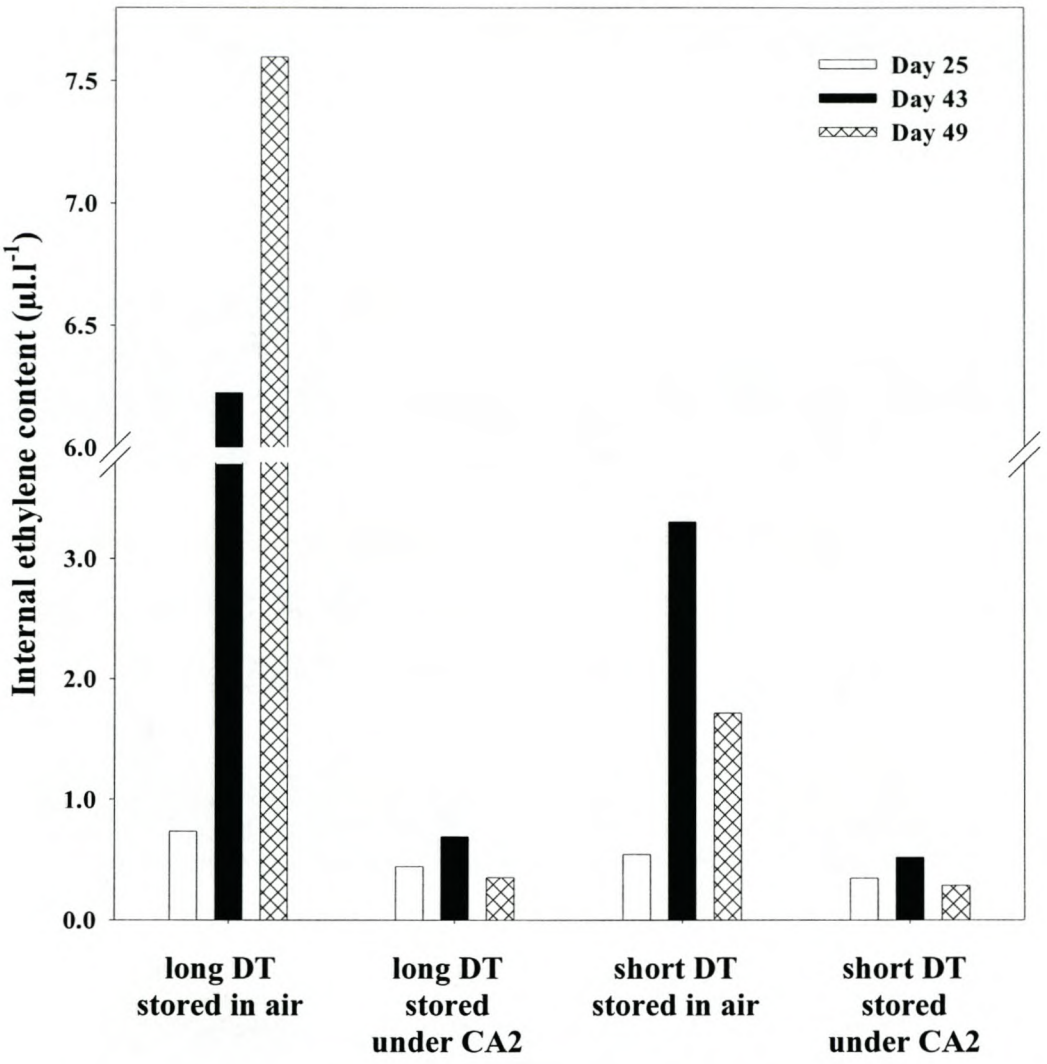

Fig. 20. Internal ethylene content ($\mu l.l^{-1}$) of 'Angeleno' plum fruit stored at -0.5°C for seven days then stored for 18 days under a long dual temperature regime (long DT) at 7.5°C or under a short dual temperature regime (short DT) (six days at -0.5°C and 12 days at 7.5°C), in either air or controlled atmosphere (CA). Thereafter the fruit were stored for 18 days at -0.5°C in air followed by a shelf life temperature of 15°C for six days. Measurements were taken initially on day seven, after 25 days storage (*LSD = 0.2373*), after 43 days storage (*LSD = 0.6110*) and after a shelf life period on day 49 (*LSD = 2.1903*).

4. ARTICLE 2: Regular Atmosphere Storage and Carbon Dioxide Shock Treatments of 'Fuerte' and 'Hass' Avocados

REGULAR ATMOSPHERE STORAGE AND CARBON DIOXIDE SHOCK TREATMENTS OF 'FUERTE' AND 'HASS' AVOCADOS

Abstract

With the increasing consumer demand for top quality avocado fruit, storage and handling technologies are being reconsidered. 'Fuerte' avocados were stored in air (RA) or treated with four carbon dioxide shock treatments over three time periods and thereafter stored in RA totalling 18 days storage. 'Hass' avocados were stored in RA or treated with two carbon dioxide shock treatments over two time periods and thereafter stored under RA, totalling 18 days storage. After storage, fruit were transferred to 20°C until eating ripe to simulate shelf life. 'Fuerte' stored under RA following CO_2 shock were generally firmer than the RA stored fruit. 'Hass' treated with CO_2 prior to RA storage, were not generally firmer than the RA stored fruit. The 96 hours CO_2 shock treated 'Fuerte' showed little pulp spot, but had of the highest grey pulp (± 32.0%), stem-end rot (± 17.2%) and vascular browning (± 45.1%). 'Hass' fruit stored under RA had the highest percentage of sound fruit (91.3%). All fruit showed significant rises in respiration and ethylene production on removal from the storage temperatures to 20°C. Maximum respiration rate for the 'Fuerte' ranged from 138 - 178 $mg.kg^{-1}.h^{-1}$ while that of 'Hass' ranged between 56 - 90 $mg.kg^{-1}.h^{-1}$. Fruit stored in CO_2 shock showed little promise as it primarily damaged the fruit. More work needs to be done using CO_2 shock treatments and refining the CO_2 levels applied to the fruit to gain improved results.

Introduction

With the excessive distances which have to be covered by South African grown avocados to reach export markets, very often fruit are travelling for more than 30 days (Couey, 1982). This very often will result in fruit arriving at the incorrect stage of maturity. The option of storing avocados at very low temperatures to restrict ripening has long since been discarded due to its susceptibility to chilling injury (Couey, 1982). This has opened the door for storage at higher temperatures of between 5 - 13°C (Kader, 1997) in combination with controlled atmospheres (CA) or CO_2

shock atmospheres (CO_2 levels which greatly exceed the initial intercellular concentrations of CO_2 are known as CO_2 shock treatments).

De Wild et al. (1999) proved that CO_2 acts as a non-competitive inhibitor in the preliminary stages of ethylene production. Kader (1997) recommends O_2 levels between 2 - 5% and CO_2 level between 3 - 10% for storage of avocados. We hypothesise that the storage of 'Fuerte' and 'Hass' avocados at 5.5°C in combination with CO_2 shock atmospheres will extend shelf life and improve quality and firmness of the fruit.

Materials and Methods

Experimental set up: 'Fuerte' avocado fruit were harvested and transported to the University of Stellenbosch by Summerfield exporters (harvest and packaging dates not known). Fruit size ranged between count 10 and count 14 (266 g - 450 g) and was intended for the local market. 'Hass' avocado fruit were harvested and transported to the University of Stellenbosch by Westfalia exporters (harvest and packaging dates not known). Fruit size was count 16 (236 g - 265 g) and was intended for the export market.

The fruit was immediately sorted on arrival ('Fuerte': 22nd June 2001 and 'Hass': 4th September 2001) and all damaged fruit were discarded. The fruit were stored at 5.5°C for 18 days simulating the approximate commercial shipping period from South Africa. Thereafter, temperatures were increased to 20°C until all fruit were eating ripe, to simulate a shelf life period in air. The treatments for 'Fuerte' were: regular atmosphere (RA) and four different carbon dioxide shock treatments over three time periods: 20% CO_2 for 24 hours (24-S1), 48 hours (48-S1) and 96 hours (96-S1); 30% CO_2 for 24 hours (24-S2), 48 hours (48-S2) and 96 hours (96-S2); 40% CO_2 for 24 hours (24-S3), 48 hours (48-S3) and 96 hours (96-S3); 50% CO_2 for 24 hours (24-S4), 48 hours (48-S4) and 96 hours (96-S4). Once treated with CO_2 shock the fruit were stored for the balance of the 18 day storage period under RA.

The treatments for 'Hass' were: regular atmosphere (RA) and two different carbon dioxide shocks treatments over two time periods: 30% CO_2 for 48 hours (48-S1) and 72 hours (72-S1) and 50% CO_2 for 48 hours (48-S2) and 72 hours (72-S2).

Fruit were placed in 25 L buckets and connected to humidified air supplied via flow boards. Flow rates were about 450 $ml.min^{-1}$ during storage and shelf life. The atmosphere composition was checked regularly and maintained within 10% of the required concentrations using an O_2 / CO_2 analyser (PBI-Dansensor, Combi Check 9800-1, Ringsted, Denmark). The 'Fuerte' experiment was a randomised block design with 14 treatments each consisting of three replications with 25 fruit each. The 'Hass' experiment was a randomised block design with five treatments each consisting of four replications with 30 fruit each.

A representative set of 20 fruit was taken initially and evaluated for firmness prior to the fruit being treated. Thereafter, five fruit per replication were removed for firmness evaluation after 18 days of storage. For the 'Fuerte' experiment during the shelf life period 14 fruit per replication and 'Hass' experiment 20 fruit per replication were removed for evaluation as they reached the eating ripe stage. This was assessed by gently squeezing the fruit by hand.

Maturity indices

Firmness. Readings were taken on opposite sides of the peeled fruit with a penetrometer (Southtrade fruit pressure tester, FT 327, Alphonsine, Italy) fitted with a 5 mm tip.

Moisture content. Moisture content was measured only initially when the fruit arrived. It was only done once as moisture content does not change much during the storage period and is used as a maturity index for harvest. Moisture content was determined by the method described by Swarts (1978). The fruit was cut in half and the pip removed. The fruit was grated at the cut surface and weighed. The sample was placed in a microwave on high for two minutes after which it was reweighed. The sample was replaced in the microwave for a further two minutes and reweighed, and the process repeated until a constant mass was achieved. The difference between the initial mass of the sample and the final mass of the sample as a percentage of the

initial mass of the sample gave the moisture content of the fruit. This was done on three fruit. For each new two minute cycle a beaker of cold water was placed in the microwave with the fruit sample, to prevent burning of the sample.

Disorders. Fruit were evaluated when eating ripe during the shelf life period. Fruit were rated for external disorders: chilling injury, black cold, Dothiorella / Colletotrichum complex (D/C) and lenticel damage. The fruit were then cut in half and allowed to stand for 10 minutes so that any internal disorders could become visible. The fruit were rated for internal disorders: pulp spot, grey pulp and vascular browning. The decay which was rated was: stem-end rot, internal anthracnose and external anthracnose. The statistics for the disorders was calculated as a percentage of the total number of fruit per replication evaluated for disorders.

Respiration rate. CO_2 levels were measured with the use of an infra-red gas analyser (IRGA) (Infra-Red Gas Analyser, S151, Kingston, Ontario), which was connected to the out flow from each of the buckets. Three fruit during the 18 days storage and one fruit during the shelf life period were enclosed in 5 L buckets to measure respiration and ethylene production. Readings were taken approximately every third day during the storage time and every day during the shelf life period. The measurements on fruit treated with CO_2 shocks were taken after the respective treatments were complete and the fruit were stored in air. This was because the CO_2 shock atmospheres had CO_2 levels greater than 0.2% (or 2000 $\mu l.l^{-1}$), which is the upper limit of the IRGA. Due to the fact that not all the treatments readings were taken on the same day's statistical analysis could not be done.

Ethylene production rate. Gas samples were taken from the out flow of each bucket, except the CO_2 shocks during treatment, on every third day during the storage period, and daily for all treatments during the shelf life period. Samples were analysed by gas chromatograph (GC Series 3000, Varian 4290 integrator, Varian Instrument Group, Palo Alto, California). Due to the fact that not all the treatments readings were taken on the same day's statistical analysis could not be done.

Internal ethylene content (IEC). A partial vacuum was applied on individual fruit with the use of a glass vacuum container with a gas tight lid and a vacuum pump

(Saltveit, 1982). Within the container the fruit was held in a flask filled with water with a septum at the point were the gas accumulates when the vacuum is applied. After the vacuum had been applied and released a sample of the extracted gas was taken with a gas tight syringe and evaluated using a gas chromatograph. An initial representative set of six fruit were evaluated on the arrival date. On removal after 18 days storage, one fruit per replication was evaluated, and another fruit per replication again after seven days at 20°C.

Ripening rates. As the fruit were removed from the shelf life period when eating ripe the number of fruit per treatment and days at 20°C until ripe were recorded.

Statistical Analysis. Analysis of variance (ANOVA) of the main effects and LSD values with a significance level of 5% were obtained using Statistical Analysis Systems (SAS). Presented data points are the means of the replications ±SE.

Results

Expt 1: 'Fuerte'

At the start of the experiment the fruit had a mean moisture content of 67.7% and were therefore stored at 5.5°C (Hardy et al., undated).

Maturity indices

Firmness. The RA stored fruit had a decrease in firmness from the initial value of 9.3 kg to 6.7 kg (Table 1). Fruit treated for 24 hours with CO_2 had firmness values which ranged from 6.2 - 8.4 kg with the fruit stored under 24-S4 being the firmest although only significantly firmer than the fruit stored under 24-S2. The fruit treated with CO_2 for 48 hours showed great variation while those fruit treated for 96 hours showed less variation with firmness values ranging between 8.6 - 9.9 kg and there was no significant difference between the treatments.

Disorders. The presence of external disorders was very restricted (Table 2). Most of the treatments were not affected by these disorders. Both D/C and lenticel damage had no significant difference between treatments. Black cold did have significant differences. However, the highest occurrence of the disorder was only 4.9%.

Internal disorders were the most prominent, affecting the fruit most severely (Table 2). The general trend for both grey pulp and vascular browning was a higher occurrence of the disorders in the fruit stored under the 96 hour CO_2 shocks. The fruit stored under 96-S2 was the most affected by grey pulp (59.0%). The RA stored fruit tended to have the lowest percentage fruit affected by vascular browning, although insignificantly so. Pulp spot, on the other hand, had the significantly highest occurrence in the RA stored fruit (45.1%) and of the CO_2 treated fruit those stored for 24 hours were more prominently affected.

The decays, stem-end rot and external anthracnose, had very high occurrence in the fruit stored under 96 hour CO_2 shock (Table 2). Fruit stored under 96-S2 had significantly higher occurrence of external anthracnose (38.5%) than all of the treatments except 96-S4 (20.5%). Internal anthracnose was far less prominent with levels reaching no higher than 5.6%.

Respiration rate. Respiration rates of the CO_2 shock treated fruit dropped between 20 - 30 $mg.kg^{-1}.h^{-1}$ from the first to the second readings (Fig. 1). Thereafter, as with the RA stored fruit, there was a relatively constant respiration rate while the fruit were stored at 5.5°C. With the onset of the shelf life period and increase in temperature to 20°C all the fruit had a sharp increase in respiration of at least 110 $mg.kg^{-1}.h^{-1}$. All the treatments had maximum respiration rate on day 20 with the fruit stored under 24-S4 reaching the highest rate (182.8 $mg.kg^{-1}.h^{-1}$). The RA stored fruit had the lowest maximum respiration rate (138.0 $mg.kg^{-1}.h^{-1}$). The remaining treatments had maximum respiration rates ranging between 143.0 - 178.6 $mg.kg^{-1}.h^{-1}$. Once all the treatments reached maximum respiration rate there was a subsequent decrease toward the end of the experiment. The slight rises again were due to decay and overripe fruit.

Ethylene production rate. The fruit stored under 24-S1 had measurable ethylene production for the first time on day 12, producing 0.4 $\mu l.kg^{-1}.h^{-1}$ (Fig. 2). The RA stored fruit had measurable ethylene production for the first time on day 19 with the onset of the shelf life period, reaching maximum production rate on day 22 (5.5 $\mu l.kg^{-1}.h^{-1}$) without displaying a distinct peak.

The fruit stored under 24-S2 had no measurable ethylene production throughout the 18 day storage period but with the onset of the shelf life period reached the highest maximum ethylene production rate on day 19 (22.0 $\mu l.kg^{-1}.h^{-1}$) (Fig. 2). Thereafter ethylene production rate slow. There was a slight increase on day 25 due to decay and overripe fruit. The remaining fruit treated with CO_2 for 24 hours showed first signs of ethylene production on day 19 without reaching a distinct maximum (3.6 - 7.0 $\mu l.kg^{-1}.h^{-1}$) except at the end of the experiment due to decay.

The fruit stored for 48 hours under CO_2 showed first signs of measurable ethylene production on day 19 with the fruit stored under 48-S4 reaching maximum production rate on day 20 (9.9 $\mu l.kg^{-1}.h^{-1}$) and the fruit stored under 48-S1 reaching maximum production rate on day 23 (18.2 $\mu l.kg^{-1}.h^{-1}$) (Fig. 2). The remaining two 48 hour CO_2 shock treated fruit displayed no distinct peak in ethylene production but the fruit stored under 48-S3 did display an increase in production rate at the end of the experiment due to decay and overripeness. The 96 hour CO_2 treated fruit had no distinct peak in ethylene production rate and the highest levels were 7.6 $\mu l.kg^{-1}.h^{-1}$ (96-S2) (Fig. 2).

Internal ethylene content (IEC). At the start of the experiment the initial IEC of the fruit was 0.1 $\mu l.l^{-1}$ (Fig. 3). The 96 hour CO_2 treated fruit generally had lower IEC levels after 18 days storage while the 48 hour CO_2 treated fruit had much variation within treatments. After the shelf life period all the treatments had an increase in IEC but there were no significant differences. Once again the general trend was the 96 hour CO_2 treated fruit having lower IEC levels.

Ripening rates. The RA stored fruit were the quickest to all reach an eating ripe state after five days at 20°C (Fig. 4). All the CO_2 treated fruit took longer to reach the same stage. The majority took seven days at 20°C to fully ripen. However, the fruit stored under CO_2 for 96 hours under S1, S2 and S3 took 9 days at 20°C for all the fruit to reach an eating ripe stage.

Expt 2: 'Hass'

At the start of the experiment the fruit had a mean moisture content of 68.3% and were therefore stored at 5.5°C (Hardy et al., undated).

Maturity indices

Firmness. On arrival the fruit had a mean firmness of 11.0 kg (Table 3). After the 18 day storage period at 5.5°C the fruit stored under 48-S2 were firmest (10.4 kg) although not significantly firmer than the fruit stored under 48-S1 and RA. The significantly softest fruit were the 72 hour CO_2 treated fruit (S1 - 5.8 kg and S2 - 6.6 kg).

Disorders. After the ripening period at 20°C the RA stored fruit had the significantly highest percentage of sound fruit (91.3%). The fruit stored under 72-S2 had the least sound fruit (47.6%).

Of the internal disorders grey pulp had insignificant differences between all the treatments with the highest percentage being only 4.9% (Table 4). Vascular browning was most prominent in the fruit stored under 72-S1 (21.3%) but insignificantly higher than the fruit stored under 72-S2 (12.2%). The remaining treatments had no significant difference and only 3.8% vascular browning occurred in the RA stored fruit (Table 4).

Stem-end rot was the most prominent of the decay disorders and the fruit stored 72-S1, 72-S2 and 48-S2 had the significantly highest percentage (30.0%, 35.1% and 34.3%, respectively) (Table 4). The fruit stored under RA were the least affected by stem-end rot having the significantly lowest percentage (3.8%). Internal anthracnose was significantly higher in the fruit stored under 72-S2 (31.4%) than all the treatments except 72-S1 (27.5%). The fruit stored under RA had significantly lower internal anthracnose (1.3%) than all the treatments except the fruit stored under 48-S1 (11.3%). External anthracnose was far less prominent with the fruit stored under RA and 48 - S1 having no occurrence of the disorder (Table 4). The remaining disorders for which the fruit were evaluated did not occur.

Respiration. The CO_2 treated fruit had respiration rates which dropped approximately 20 $mg.kg^{-1}.h^{-1}$ from the first to the second readings (Fig. 5). Thereafter, as with the RA stored fruit, there was a relatively constant respiration rate of ± 10 $mg.kg^{-1}.h^{-1}$ while the fruit were held at 5.5°C. With the onset of the shelf life period and subsequent increase in temperature to 20°C all the fruit had a sharp increase in respiration rate of at least 60 $mg.kg^{-1}.h^{-1}$. The fruit stored under 48-S2 had the highest maximum respiration rate on day 20 (90.1 $mg.kg^{-1}.h^{-1}$). Thereafter, the fruit stored under 48-S1 had a maximum respiration rate slightly lower on day 21 (83.6 $mg.kg^{-1}.h^{-1}$). The fruit which had the lowest maximum respiration rate were the fruit stored under 72-S2 (69.6 $mg.kg^{-1}.h^{-1}$) on day 20. After the sharp increase in respiration all the fruit showed a slight drop off and stabilising of respiration rate.

Ethylene production rate. As with respiration rate, most of the treatments had a relatively distinct peak and a drop off in ethylene production towards the end of the experiment (Fig. 6). The fruit stored under 48-S1 had the highest ethylene production rate on day 21 (50.2 $ul.kg^{-1}.h^{-1}$). The remaining treatments all reached a peak in ethylene production on either day 20 or 21 with the lowest peak by the fruit stored under 72-S2 on day 21 (22.6 $ul.kg^{-1}.h^{-1}$) and this was not a very distinct peak.

Internal ethylene (IEC). There was large variation in IEC between treatments from the initial IEC of 0.2 $\mu l.l^{-1}$ after 18 days storage (Fig. 7). The fruit stored under 48-S1 and 72-S1 had the highest IEC (6.2 and 5.5 $\mu l.l^{-1}$, respectively) but insignificantly higher than the fruit stored under RA and 72-S2. After the shelf life period at 20°C there was a sharp increase in IEC from the first evaluation time with levels ranging from 107.9 $ul.kg^{-1}.h^{-1}$ to 210.4 $ul.kg^{-1}.h^{-1}$. There were no significant differences between the treatments.

Ripening rates. The fruit stored under RA ripened the quickest, being fully eating ripe after 5 days at 20°C (Fig. 8). The CO_2 treated fruit all ripened slower and were all fully eating ripe after 9 days at 20°C.

Discussion and Conclusion

Firmness. During the 'Fuerte' experiment the CO_2 treated fruit generally had firmer fruit that the fruit stored under RA but the interaction between CO_2 concentration and length of exposure of the fruit to CO_2 shock levels was significant (0.0008), thus the main effects cannot be discussed (Table 5). At this point it must be said that during the 18 day storage period of the 'Hass' experiment the cold room in which the fruit were stored under 72-S1 and 72-S2 was faulty. A Tinytag Plus temperature sensor revealed that the room was cooling to about 7.5°C instead of the supposed 5.5°C for much of the 18 days. With this in mind the interaction between CO_2 concentration and time was insignificant (Table 6). Thus, length of exposure had the main effect as the 72 hour CO_2 shock treated fruit were significantly softest. If the 72 hour CO_2 treated fruit were disregarded due to the faulty cold room the remaining 48 hour CO_2 treated fruit did not affect fruit firmness as would be expected as there was no significant difference compared to the RA stored fruit.

Lelièvre et al. (1997) found that fruit softening is the ripening process which is the most affected by ethylene. This is supported by the fact that polygalacturonase (PG), a key enzyme involved in cell wall hydrolysis (Dong et al., 2001), is regulated by ethylene (Sitrit and Bennett, 1998). Along with PG, pectinesterase (PE) (Dong et al., 2001) and cellulase (Awad and Young, 1979) are also involved in cell wall hydrolysis. It has been found that PG and cellulase activity increases while PE decreases during fruit ripening (Awad and Young, 1979). The early increase in cellulase activity prior to increased PG activity has led some researchers to believe that cellulase is responsible for the early stages of avocado fruit softening and PG for final fruit softening (Bower and Cutting, 1988). The fact that increased CO_2 levels have an inhibitory effect on both the enzymes involved in ethylene production (De Wild et al., 1999) would explain why the fruit retain firmness under these conditions. This effect has been found in CA storage of apples with O_2 levels between 2.5 and 4.0% and CO_2 levels up to 3.2%, resulting in fruit firmer than the control fruit (Bender, 1989; Knee as reported by Kader, 1986)

During CO_2 shock treatment there is a build up of CO_2 within the fruit through diffusion which consequently helps the fruit retain firmness by restricting sugar loss

and overripening (Salisbury and Ross, 1991). Nicolas et al. (1989) found that CO_2 shock treatment of kiwi fruit had a significant effect in delaying fruit softening and this effect increased with the number of exposures to high CO_2.

Disorders. During the 'Fuerte' experiment compared to the fruit treated with CO_2 the fruit stored under RA performed indifferently but most noticeable were the extremely high levels of pulp spot (Table 2). For both pulp spot and grey pulp the interaction between CO_2 concentration and length of exposure to high CO_2 concentration was significant at the 5% level, thus the main effects cannot be discussed (Table 5). Vascular browning did not have a significant interaction and thus the extent of the disorder was significantly influenced by both length of exposure and CO_2 concentration. Thus the longer exposure time of 96 hours to CO_2 shock levels caused more vascular browning. The 20% and 50% concentrations of CO_2 generally lowered the incidence of vascular browning while the two intermediate concentrations generally increased the incidence. Stem-end rot and external anthracnose did not have a significant interaction thus the main influence was time as the extent of injury increased with the length of exposure to the high CO_2 levels. The remaining disorders had no significance with regard to any of the parameters as the incidence of these disorders was very low.

In general 'Hass' avocados are known to be far less susceptible to disorders than most cultivars. This is most clearly seen as the RA stored fruit had the highest percentage of sound fruit and of the lowest incidence of each mentioned disorder (Table 4). There was no significant interaction between length of exposure and concentration of CO_2 for any of the disorders (Table 6). The longer the exposure time to the higher CO_2 and the higher the CO_2 level, the fewer sound fruit there were. Stem-end rot was affected by the higher CO_2 levels while vascular browning and internal anthracnose were affected by the longer exposure times to the high CO_2 levels. Once again, this was possibly due to the faulty cold room during storage.

It is critical that avocados are stored under low ethylene conditions due to the role that ethylene plays in chilling injury (White et al., 2001). This is highlighted by the fact that avocados are most sensitive to chilling injury at the climacteric peak (Donkin, 1995). External chilling injury commonly occurs in the form of blackening and

pitting of the exocarp while internally discolouration of the mesocarp is characteristic (Couey, 1982). Swarts (1984) identified two types of internal chilling injury: pulp spot, which is associated with the cut ends of vascular bundles resulting in grey spots and grey pulp which is a grey to brown discolouration of the mesocarp. Polyphenol oxidase (PPO) is the enzyme which catalyses the grey and browning reactions of the mesocarp (Bower and Cutting, 1988).

Truter et al. (1992) found that CA treated 'Fuerte' avocados had the lowest PPO activity during storage but the activity increased higher than the control fruit during the ripening period but the CA treated fruit still had fewer disorders than the control fruit. This was due to a change in PPO activity before softening (Cutting et al., 1990). Thus, where browning had occurred, activity was low and vice versa for the CA treated fruit (Truter et al., 1992). 'Fuerte' avocados treated with an initial CO_2 concentration of 5% which increased to 35% three days after harvest, followed by normal storage, prevented anthracnose, chilling injury, grey flesh and pulp spot (Truter and Eksteen, 1987). Furthermore it has been found that elevated CO_2 levels prior to storage can make fruit more resistant to *Colletotrichum gloeosporioides* by increased levels of antifungal diene (Prusky et al., as reported by Lange and Kader, 1997). These stress levels can, however, cause damage to the fruit (Lange and Kader, 1997).

Respiration rate. The relationship between temperature and ripening is expressed as the temperature coefficient (Q_{10}), which describes the increase in respiration for a 10°C rise in temperature (Kader, 2002). An increase of 10°C above the optimum storage temperature for most non-chilling sensitive commodities will result in a two to three fold increase in respiration and thus deterioration. This would explain the distinct rises in respiration rate for the all the fruit regardless of treatment on exposure to 20°C during the shelf life period (Fig. 1).

The rise in respiration during ripening of certain fruit without an increase in temperature was named the respiratory climacteric by Kidd and West (as reported by Blanke, 1991). For avocado fruit this is facilitated by detachment from the tree (Blanke, 1991). The CO_2 shock treatments result in a progressive reduction in fruit respiration (Blanke, 1991). This is done by slowing fruit metabolism, mitochondrial

activity and respiration after succinate dehydrogenase is inhibited causing an irreversible accumulation of succinate (Blanke, 1991). It can, however, be seen from this study that the respiratory peaks of all the CO_2 shock treated fruit were generally not delayed and did not peak lower when compared to the RA stored fruit. In contrast to this Ong (as reported by Kader, 1989) found that the respiration rates of 'Bartlett' pears steadily decreased with increasing levels of CO_2 concentration in air after four days at 20°C.

Ethylene production rate. During the 'Fuerte' experiment most of the CO_2 treated fruit had higher ethylene production rates than the fruit stored under RA. In contrast, during the 'Hass' experiment, the fruit stored under 48 - S1 were the only CO_2 treated fruit to have a higher ethylene production rate than the fruit stored under RA. Depending on the commodity and concentration elevated CO_2 levels can reduce, promote or have no effect on ethylene production (Kader, 1986). Cheverry et al. (1988) demonstrated that treatment of 'Fuerte' avocados with 20% CO_2 inhibited climacteric ethylene production.

It was originally believed that CO_2 acted as a competitive inhibitor of ethylene action (Burg and Burg, 1967). It was, however, proven that increased CO_2 levels act as a non-competitive inhibitor of ethylene action affecting either of the two main enzymes involved in ethylene production namely ACC synthase or ACC oxidase (Gorny and Kader, 1993). This was confirmed by treatment of 'Bartlett' pears with 1% and 20% CO_2. As the level of CO_2 increased there was a decrease in ACC synthase activity (Chavez-Franco and Kader, 1993).

Internal ethylene (IEC). It would have been expected that the CO_2 treated fruit would have had less IEC at each of the evaluation times as was found by Van Eeden et al. (1990) with treatment of 'Hass' avocados with 20% CO_2 for three days at 5°C. This was not the case. For the 'Fuerte' experiment the interaction between length of exposure to the high CO_2 levels and the concentration of CO_2 was significant after 18 days storage, thus the main effects cannot be discussed. After 25 days storage none of the parameters were significant at the 5% level. During the 'Hass' experiment, only after 18 days storage did one of the parameters have a significant

influence, viz. the concentration of CO_2. This means that with the increase in CO_2 concentration there was a decrease in IEC.

Ripening rates. Ethylene is a hormone directly involved in fruit ripening (Lelièvre, 1997). This is most evident when comparing the ethylene production rate, IEC and ripening rates of the fruit treated with CO_2 for 96 hours during the 'Fuerte' experiment. The ethylene production rate of these fruit stayed low with the onset of the shelf life period when compared to the other treatments and no distinct peak was reached. A similar pattern develops for IEC with the same fruit having lower levels after both 18 days of storage and again after the shelf life period (Fig. 2). This relationship between ethylene and fruit ripening is then displayed in Fig. 3 where the same 96 hour CO_2 treated fruit generally had slower fruit ripening and generally also had firmer fruit after the 18 day storage period.

A similar pattern was seen on the fruit stored under 48-S2 and 48-S4 during the 'Fuerte' experiment. These two treatments had of the firmest fruit after 18 days storage and at the same evaluation time had of the lowest IEC. The fruit treated with CO_2 shocks did have a positive influence on delaying ripening of both cultivars when compared to the fruit stored under RA.

When considering the proposed hypothesis the conditions for the CO_2 treated fruit were met to varying degrees when compared to the RA treated fruit with regard to extending shelf life and improving firmness. However, in conclusion, the use of CO_2 shock atmospheres, as has been shown in the data, shows little promise as it seems to primarily damage the fruit. Less extreme levels of CO_2 or shorter exposure times could prove to be more applicable to the storage of avocados.

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Table 1

Firmness (kg) of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or four carbon dioxide shock treatments over three time periods and stored for the balance of the 18 days in air.

	Firmness (kg)	
Initial	9.3	
RA	6.7	cd[z]
24 - S1[y]	6.8	cd
24 - S2	6.2	d
24 - S3	7.5	bcd
24 - S4	8.4	abc
48 - S1	5.8	d
48 - S2	9.6	ab
48 - S3	6.0	d
48 - S4	10.0	a
96 - S1	9.9	a
96 - S2	9.0	ab
96 - S3	9.7	a
96 - S4	8.6	abc
LSD	*2.1357*	

[z] Means separation within columns using least significant differences (0.05)

[y] *Period of CO_2 shock (24, 48 and 96 hrs) at 20% (S1), 30% (S2), 40% (S3) and 50% (S4) in air.*

Table 2

Internal and external disorders (%) of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or four carbon dioxide shock treatments over three time periods and stored for the balance of the 18 days in air. The fruit were then stored at 20°C until eating ripe and were then evaluated.

	Dothiorella/ Colletotrichum complex (%)	Black cold (%)	Lenticels (%)	Pulp spot (%)	Grey pulp (%)	Vascular browning (%)	Stem-end rot (%)	Anthracnose Internal (%)	Anthracnose External (%)
RA	0.0 ns[z]	0.0 b	0.0 ns	45.1 a	17.7 cd	7.8 e	2.0 c	3.9 ab	3.9 b
24 – S1[y]	0.0	0.0 b	0.0	25.6 b	18.0 cd	20.5 de	5.1 bc	0.0 b	7.7 b
24 – S2	5.1	0.0 b	0.0	25.6 b	23.1 bcd	33.3 abcd	5.1 bc	0.0 b	5.1 b
24 – S3	0.0	2.4 ab	0.0	12.7 cd	20.0 bcd	28.0 bcd	5.3 bc	2.6 ab	16.9 b
24 – S4	0.0	2.6 ab	0.0	7.7 de	14.7 d	22.4 cde	4.4 bc	0.0 b	7.4 b
48 – S1	5.1	0.0 b	0.0	21.6 bc	21.2 bcd	28.8 bcd	9.8 abc	2.1 ab	9.3 b
48 – S2	0.0	4.9 a	0.0	4.9 de	20.8 bcd	27.8 bcd	4.2 c	5.6 a	13.9 b
48 – S3	5.3	0.0 b	2.6	5.1 de	34.0 bc	44.7 ab	2.8 c	0.0 b	7.7 b
48 – S4	0.0	2.6 ab	0.0	0.0 e	12.8 d	20.5 de	7.7 abc	0.0 b	12.8 b
96 – S1	0.0	2.4 ab	0.0	0.0 e	14.7 d	39.2 abc	14.8 abc	4.8 ab	17.4 b
96 – S2	0.0	0.0 b	0.0	0.0 e	59.0 a	51.3 a	20.5 a	2.6 ab	38.5 a
96 – S3	0.0	0.0 b	0.0	2.6 de	35.9 b	51.3 a	18.0 ab	2.6 ab	18.0 b
96 – S4	0.0	0.0 b	2.6	0.0 e	18.0 cd	38.5 abcd	15.4 abc	0.0 b	20.5 ab
LSD	*6.231*	*4.4698*	*2.9227*	*10.897*	*17.778*	*18.149*	*13.704*	*5.1793*	*19.494*

[z] Means separation within columns using least significant differences (0.05)

[y] *Period of CO_2 shock (24, 48 and 96 hrs) at 20% (S1), 30% (S2), 40% (S3) and 50% (S4) in air.*

Table 3

Firmness (kg) of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or two carbon dioxide shock treatments over two time periods and stored for the balance of the 18 days in air.

	Firmness (kg)	
Initial	11.0	
RA	10.1	a[z]
48-S1[y]	10.1	a
48-S2	10.4	a
72-S1	5.8	b
72-S2	6.6	b
LSD	*1.8902*	

[z] Means separation within columns using least significant differences (0.05)

[y] *Period of CO_2 shock (48 and 72 hrs) at 30% (S1) and 50 % (S2) in air.*

Table 4

Internal and external disorders (%) of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or two carbon dioxide shock treatments over two time periods and stored for the balance of the 18 days in air. The fruit were then stored at 20°C until eating ripe and were then evaluated.

	Sound fruit (%)	Grey pulp (%)	Vascular browning (%)	Stem-end rot (%)	Anthracnose (%)	
					Internal	External
RA	91.3 a[z]	1.3 ns	3.8 b	3.8 c	1.3 d	0.0 ns
48-S1[y]	75.0 b	1.3	6.3 b	17.5 b	11.3 cd	0.0
48-S2	61.2 c	2.4	3.4 b	34.3 a	17.6 bc	1.1
72-S1	57.5 cd	3.8	21.3 a	30.0 a	27.5 ab	5.0
72-S2	47.6 d	4.9	12.2 ab	35.1 a	31.4 a	4.0
LSD	*12.888*	*5.7667*	*9.3891*	*11.758*	*11.620*	*6.1675*

[z] Means separation within columns using least significant differences (0.05)

[y] *Period of CO_2 shock (48 and 72 hrs) at 30% (S1) and 50 % (S2) in air.*

Table 5

Interaction between length of exposure to high CO_2 levels and concentration of CO_2 for firmness and internal and external disorders of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or four carbon dioxide shock treatments over three time periods and stored for the balance of the 18 days in air and evaluated for firmness. The fruit were then stored at 20°C until eating ripe and were then evaluated for disorders.

		Dothiorella /								
		Colletotrichum	Black	Lenticel	Pulp	Grey	Vascular	Stem-end	Anthracnose	
	Firmness	complex	cold	damage	spot	pulp	browning	rot	Internal	External
Time	0.0006	0.2719	0.5460	0.6127	0.0001	0.0207	0.0009	0.0027	0.3403	0.0135
Concentration	0.0554	0.7176	0.8266	0.5807	0.0013	0.0023	0.0430	0.9864	0.2793	0.5773
Time*Concentration	0.0008	0.2739	0.1921	0.3564	0.0205	0.0212	0.6733	0.9092	0.2982	0.3943

Table 6

Interaction between length of exposure to high CO_2 levels and concentration of CO_2 for firmness and internal and external disorders of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or two carbon dioxide shock treatments over two time periods and stored for the balance of the 18 days in air and evaluated for firmness. The fruit were then stored at 20°C until eating ripe and were then evaluated for disorders.

		Sound	Grey	Vascular	Stem-end	Anthracnose	
	Firmness	fruit	pulp	browning	rot	Internal	External
Time	0.0001	0.0041	0.2451	0.0034	0.1375	0.0042	0.1115
Concentration	0.4544	0.0193	0.5908	0.0932	0.0229	0.2558	0.9940
Time*Concentration	0.6900	0.6669	1.0000	0.3651	0.1900	0.7781	0.6483

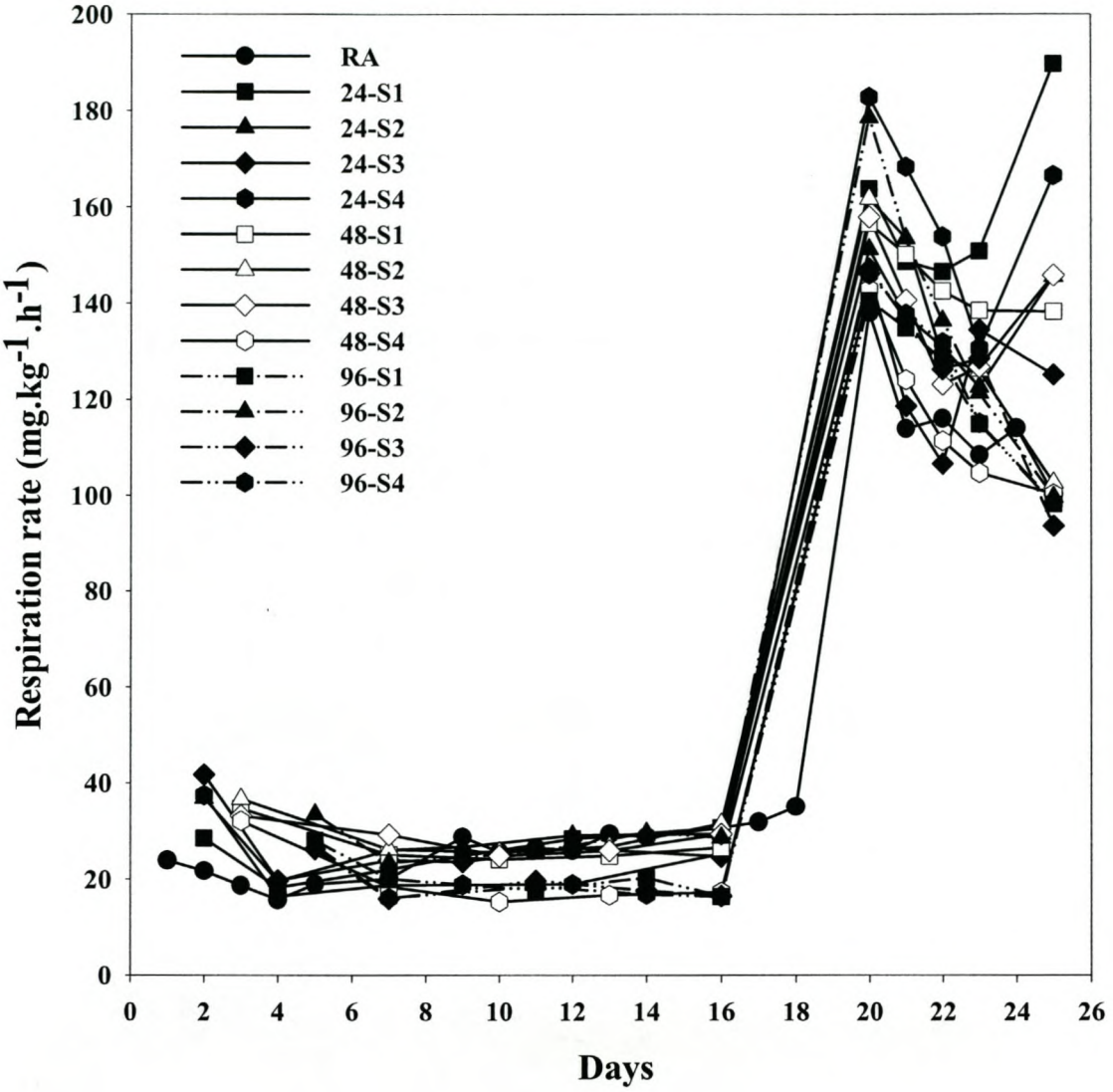

Fig. 1. Respiration rate (CO_2 evolved in $mg.kg^{-1}.h^{-1}$) of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or four carbon dioxide shock treatments over three time periods and stored for the balance of the 18 days in air. The fruit were then stored at 20°C until eating ripe.

Period of CO_2 shock (24, 48 and 96 hrs) at 20% (S1), 30% (S2), 40% (S3) and 50% (S4) in air.

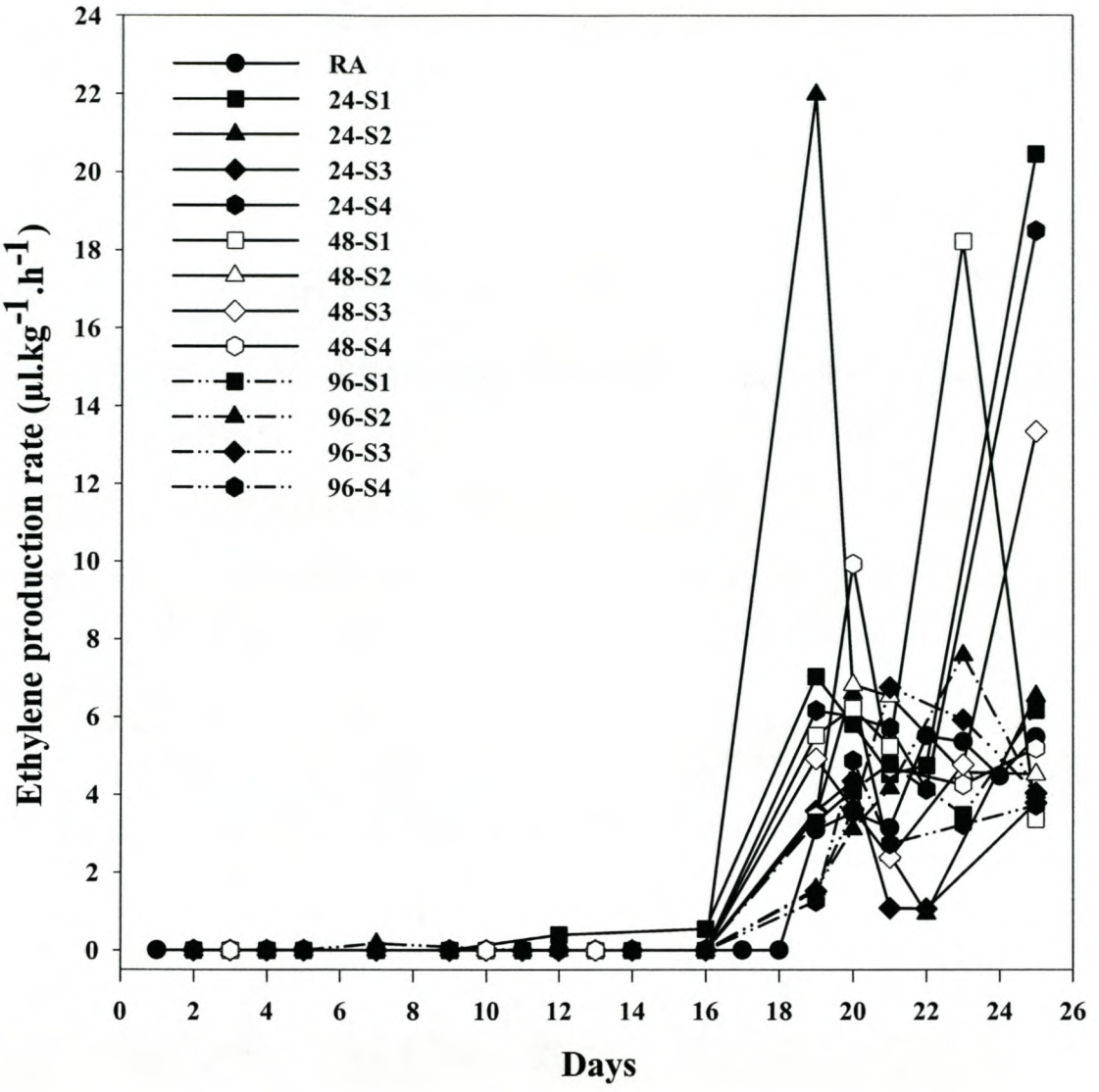

Fig. 2. Ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or four carbon dioxide shock treatments over three time periods and stored for the balance of the 18 days in air. The fruit were then stored at 20°C until eating ripe.

Period of CO_2 shock (24, 48 and 96 hrs) at 20% (S1), 30% (S2), 40% (S3) and 50% (S4) in air.

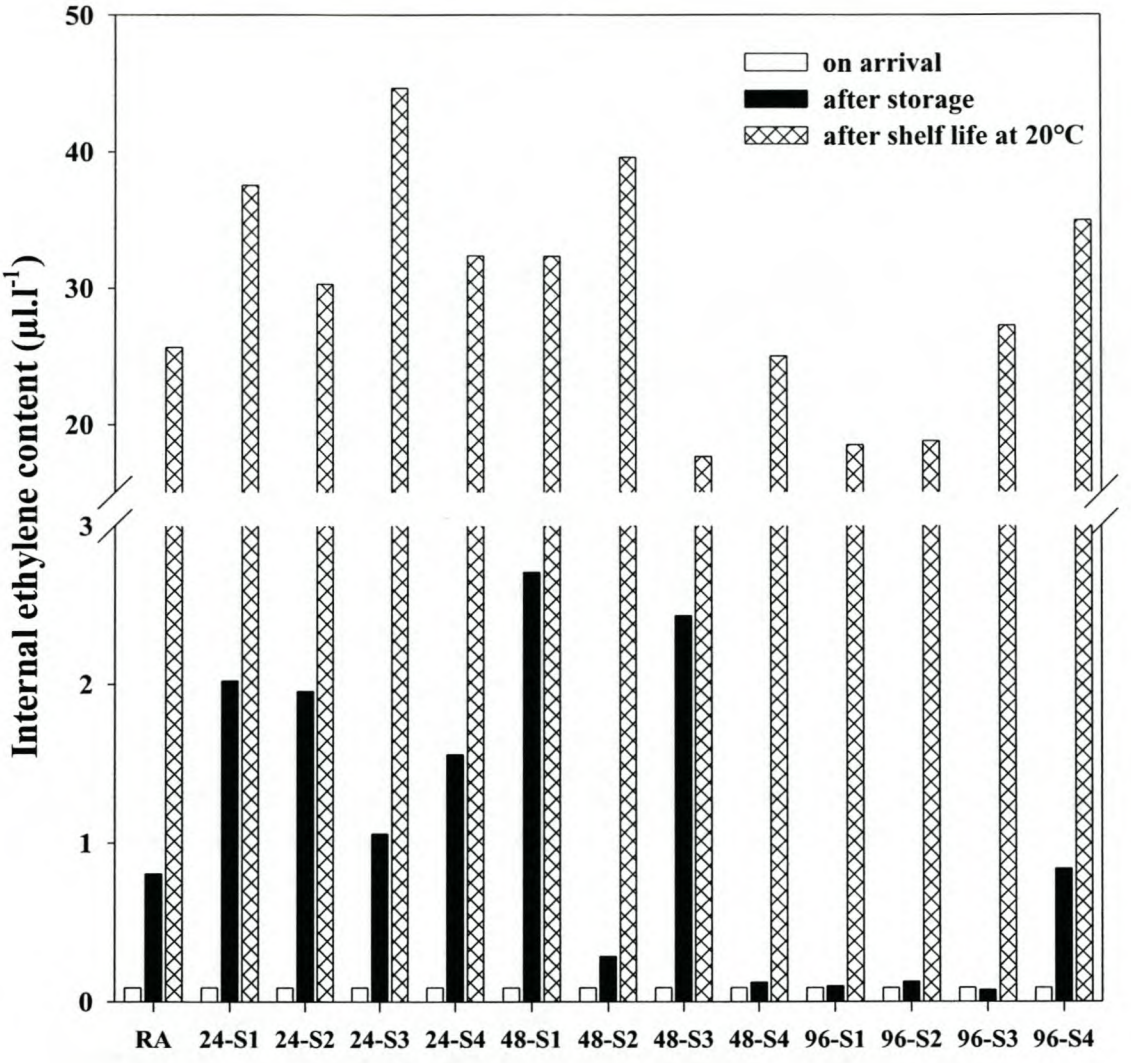

Fig. 3. Internal ethylene content ($\mu l.l^{-1}$) of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or four carbon dioxide shock treatments over three time periods and stored for the balance of the 18 days in air. The fruit were then stored at 20°C until eating ripe. Measurements were taken initially on arrival, after 18 days storage (*LSD = 1.3618*) and after the shelf life period on day 25 (*LSD = 28.155*).

Period of CO_2 shock (24, 48 and 96 hrs) at 20% (S1), 30% (S2), 40% (S3) and 50% (S4) in air.

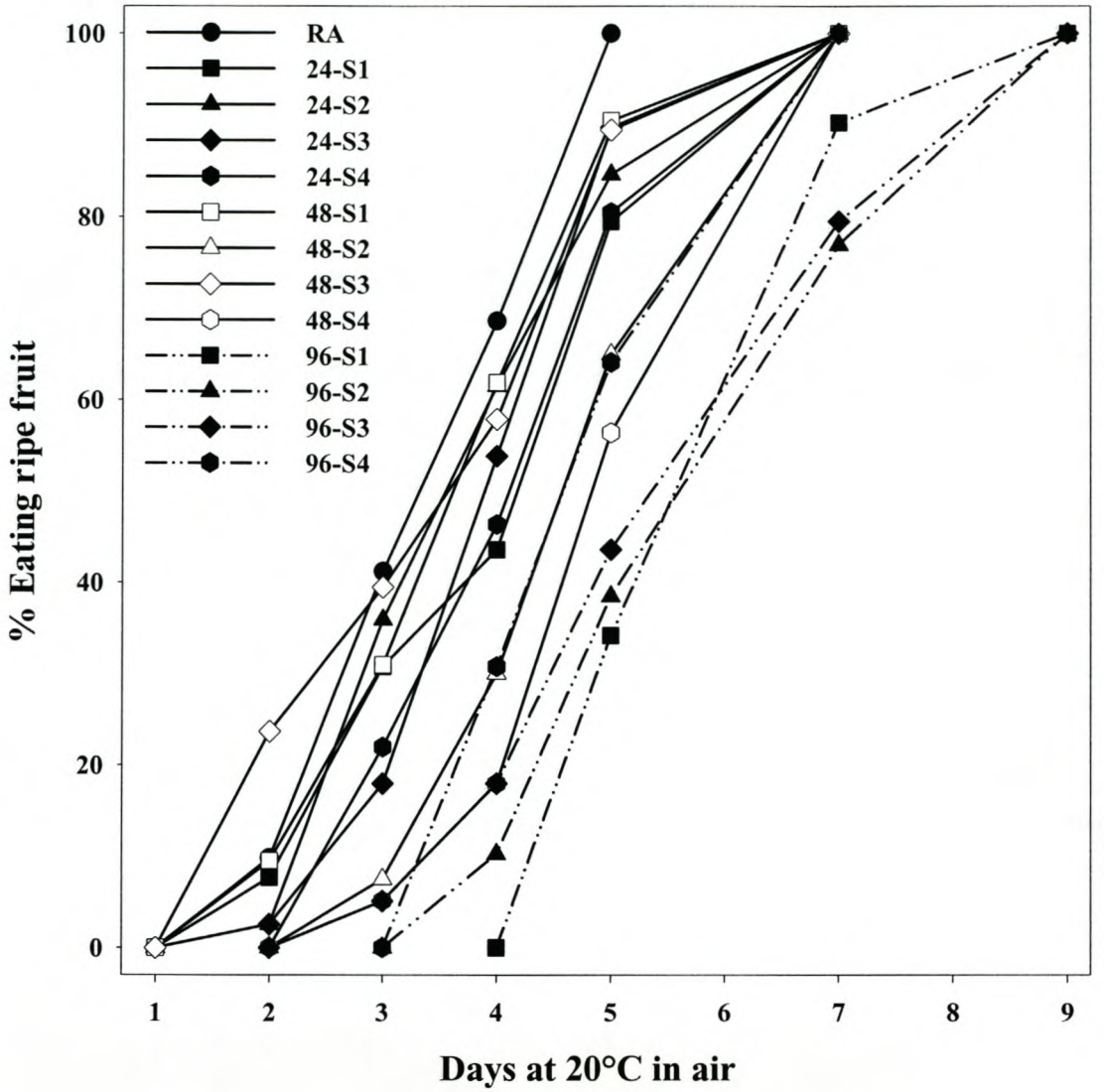

Fig. 4. Ripening rates of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or four carbon dioxide shock treatments over three time periods and stored for the balance of the 18 days in air. The fruit were then stored at 20°C until eating ripe and the percentages recorded.

Period of CO_2 shock (24, 48 and 96 hrs) at 20% (S1), 30% (S2), 40% (S3) and 50% (S4) in air.

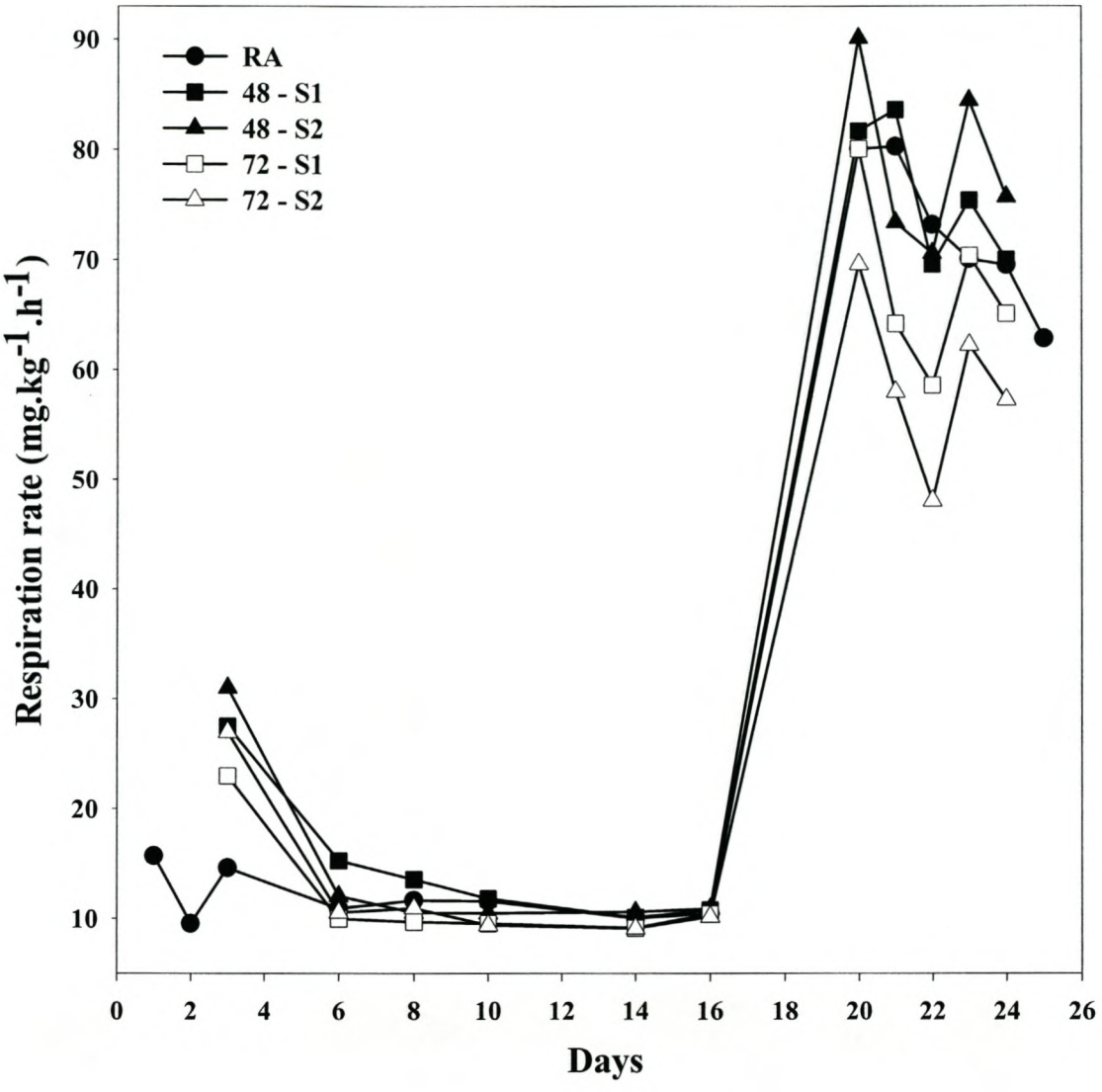

Fig. 5. Respiration rate (CO_2 evolved in $mg.kg^{-1}.h^{-1}$) of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or two carbon dioxide shock treatments over two time periods and stored for the balance of the 18 days in air. The fruit were then stored at 20°C until eating ripe.

Period of CO_2 shock (48 and 72 hrs) at 30% (S1) and 50 % (S2) in air.

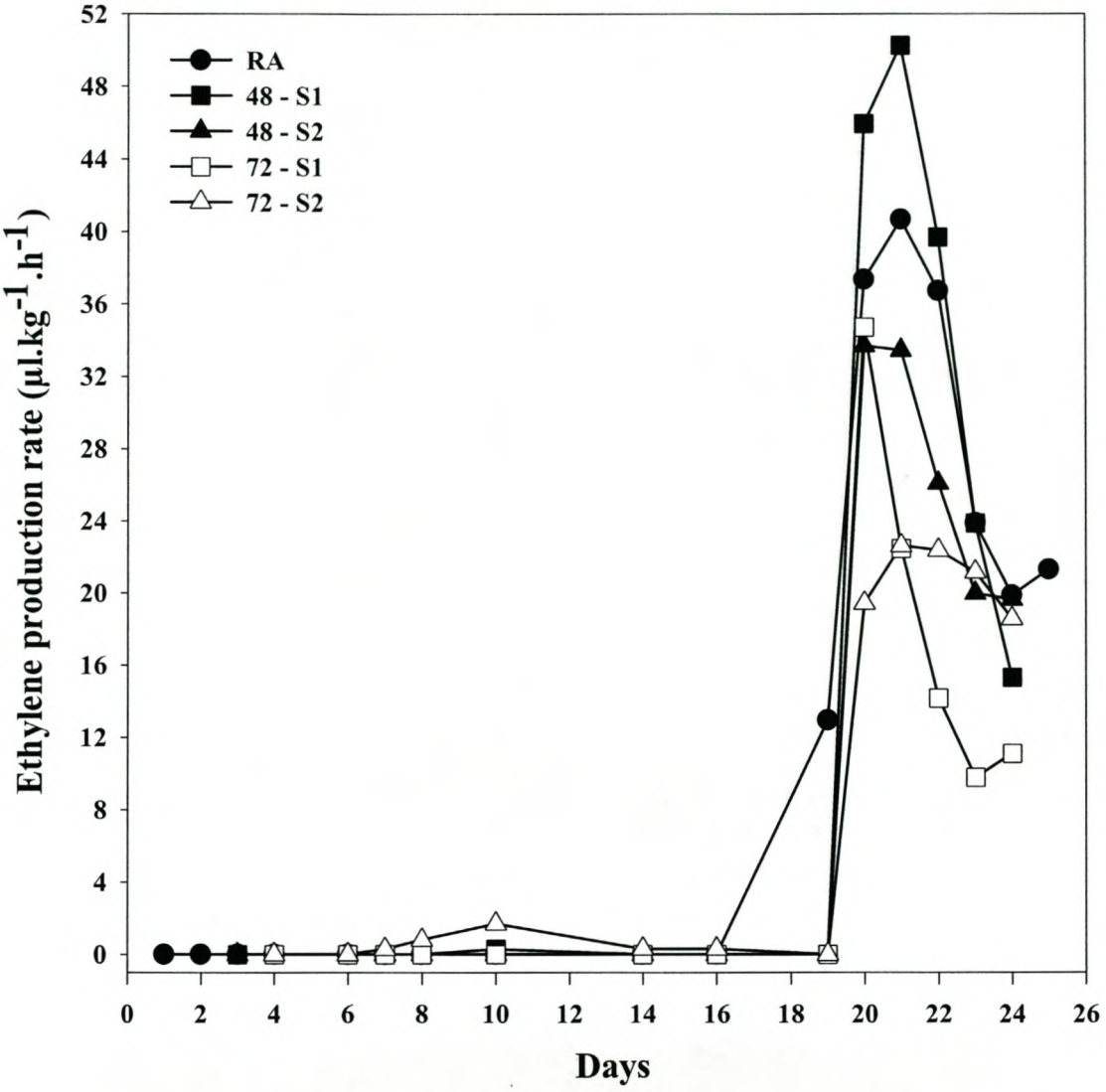

Fig. 6. Ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or two carbon dioxide shock treatments over two time periods and stored for the balance of the 18 days in air. The fruit were then stored at 20°C until eating ripe.

Period of CO_2 shock (48 and 72 hrs) at 30% (S1) and 50 % (S2) in air.

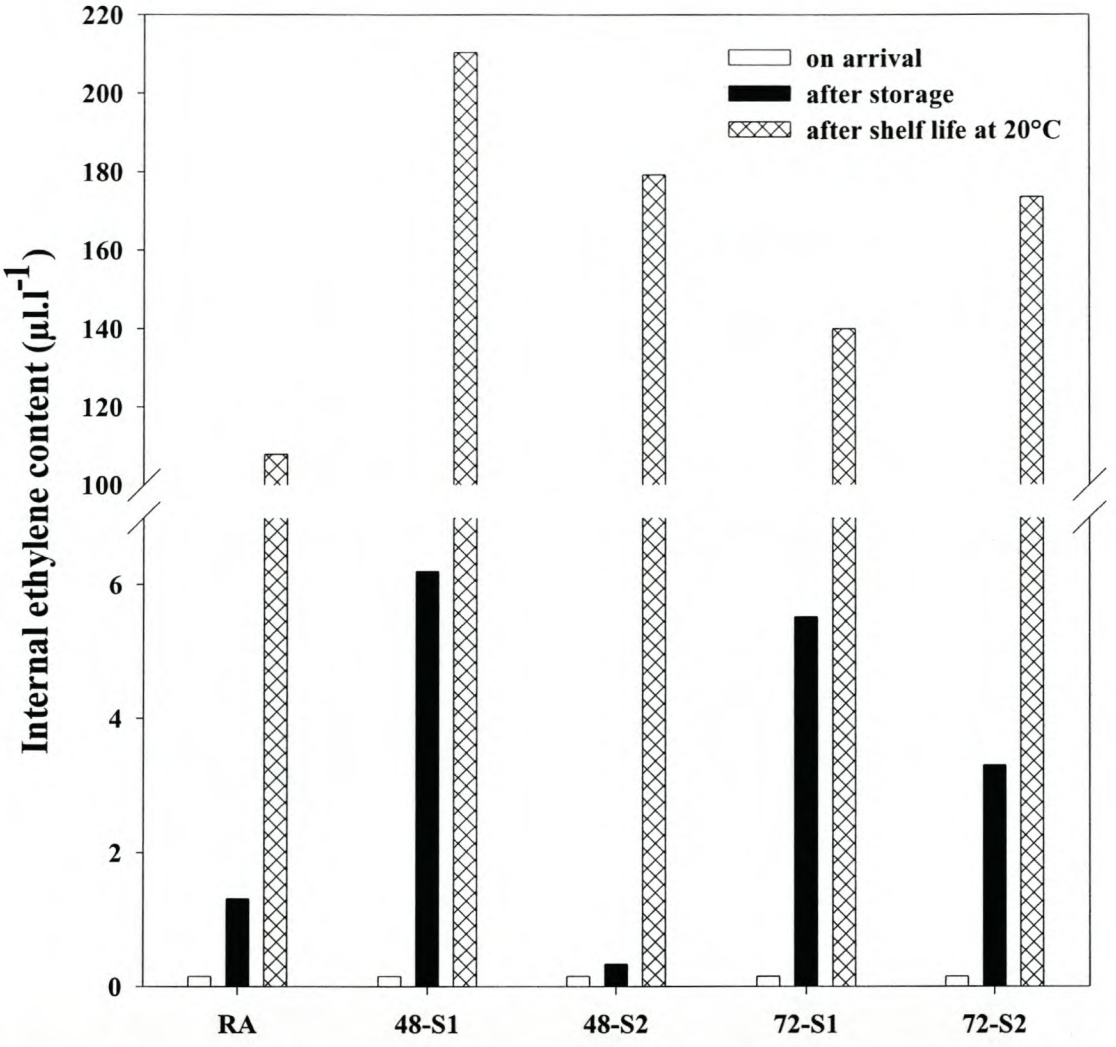

Fig. 7. Internal ethylene content ($\mu l.l^{-1}$) of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or two carbon dioxide shock treatments over two time periods and stored for the balance of the 18 days in air. The fruit were then stored at 20°C until eating ripe. Measurements were taken initially on arrival, after 8 days storage (*LSD = 5.0133*) and after the shelf life period on day 25 (*LSD = 147.18*).

Period of CO_2 shock (48 and 72 hrs) at 30% (S1) and 50 % (S2) in air.

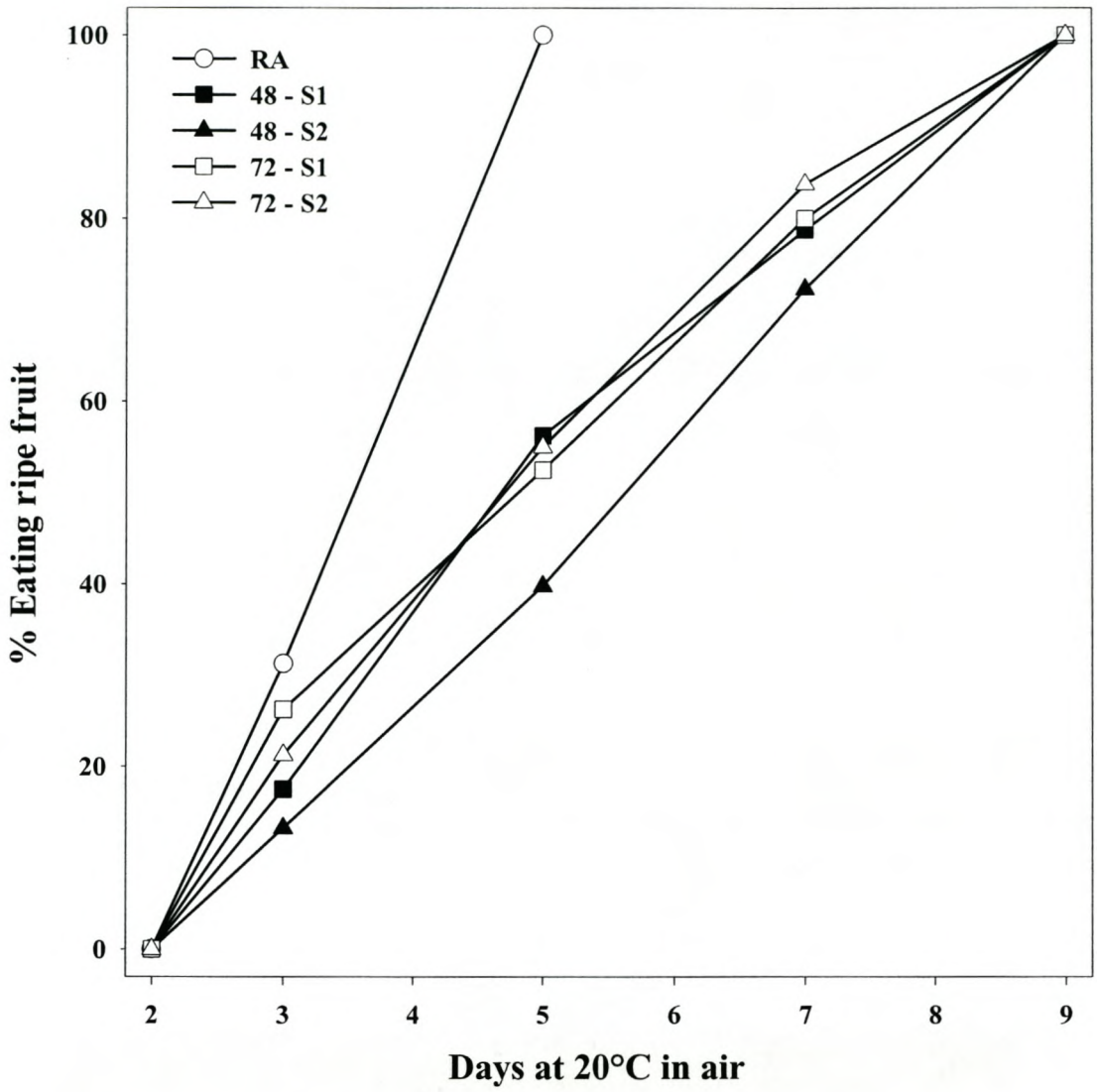

Fig. 8. Ripening rates of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or two carbon dioxide shock treatments over two time periods and stored for the balance of the 18 days in air. The fruit were then stored at 20°C until eating ripe and the percentages recorded.

Period of CO_2 shock (48 and 72 hrs) at 30% (S1) and 50 % (S2) in air.

5. ARTICLE 3: Regular and Controlled Atmosphere Storage of 'Fuerte' and 'Hass' Avocados with and without 1-methylcyclopropene.

REGULAR AND CONTROLLED ATMOSPHERE STORAGE OF 'FUERTE' AND 'HASS' AVOCADOS WITH AND WITHOUT 1-METHYLCYCLOPROPENE.

Abstract

With the increasing consumer demand for top quality avocado fruit, storage and handling technologies are being reconsidered. During season one 'Fuerte' avocados were stored under regular atmosphere (RA) or controlled atmosphere (CA) for 18 days. 'Hass' avocados were stored under RA, with 1-methylcyclopropene (1-MCP) (RA 1-MCP) or without 1-MCP and stored under CA, with 1-MCP (CA 1-MCP) or without 1-MCP. After storage, fruit were transferred to 20°C until eating ripe to simulate shelf life. 'Fuerte' stored under CA were about 3 kg firmer (9.8 kg) than the fruit stored under RA after 18 days. 'Hass' fruit stored under CA were more than 2 kg firmer (12.2 kg) than the fruit stored under RA, after 18 days storage. 'Hass' treated with CA 1-MCP had a firmness value of 11.5 kg. 'Fuerte' stored under CA had no incidence of pulp spot (0%), low levels of internal anthracnose (5.1%), and slightly higher grey pulp and external anthracnose (both 10.3%) when compared to the fruit stored under RA. 'Hass' stored under RA had the highest percentage of sound fruit (91.3%) while the RA 1-MCP treated fruit were 85.0% sound. All fruit had sharp increases in respiration rate, ethylene production rate and internal ethylene content on removal from the storage temperatures to 20°C. The fruit stored under RA 1-MCP, CA or CA 1-MCP all restricted ripening of the fruit compared to the fruit stored under RA. During season two the 'Fuerte' and 'Hass' avocado fruit were stored under RA, with and without 1-MCP and under CA, with and without 1-MCP. The fruit treated with CA, RA 1-MCP or both had positive results in delaying softening of the fruit compared to the fruit stored under RA. The fruit treated with CA 1-MCP were the firmest after 28 days storage ('Fuerte': 9.9 kg and 'Hass': 11.9 kg). The same treatment had positive results in restricting disorders of 'Fuerte' avocados but were less effective on the 'Hass' avocados. All the fruit had strong increases in respiration rate, ethylene production rate and internal ethylene content with the increase in temperature to 20°C. The fruit stored under RA had the fastest ripening pattern. There was a case of uneven ripening with the fruit stored under RA 1-MCP. Fruit stored under CA, 1-MCP or both showed positive results but

considering the cost of CA, 1-MCP may be regarded as the key to future long-term storage of avocados.

Introduction

Over the years it has become very common for South African grown avocados to arrive at the overseas market at the incorrect stage of maturity (ie. under ripe or overripe). This is partly due to the fact that very often fruit are in transit for more than 30 days, from the date of harvest, before reaching the export market (Couey, 1982). The option of storing avocados at very low temperatures to restrict ripening has long since been discarded due to susceptibility of the fruit to chilling injury (Couey, 1982). This has opened the door for storage at higher temperatures of between 5 - 13°C (Kader, 1997) in combination with controlled atmospheres (CA) or 1-methylcyclopropene (1-MCP). Kader (1997) recommends O_2 levels between 2 - 5% and CO_2 level between 3 - 10% for storage of avocados.

1-MCP was proven by De Wild et al. (1999) and Rupasinghe et al. (2000a) to be a competitive inhibitor of ethylene at the active binding site, therefore blocking ethylene action and delaying ripening of the fruit. The binding of 1-MCP to the ethylene receptor is more efficient than ethylene itself, making 1-MCP treatments effective in very low dosages in the $nl.l^{-1}$ range (Rupasinghe et al., 2000a). Furthermore, 1-MCP blocks the normal feedback regulation of ethylene and therefore delays ripening of the fruit (Golding et al., 1998). The application of 1-MCP must be done before autocatalytic ethylene production is measurable for it to be most effective otherwise it will be too late to affect the progress of the climacteric (Golding et al., 1998).

It has also been found that treatment at lower temperatures requires higher concentrations of 1-MCP for the same result (Sisler et al., 1996). For this reason treatments with 1-MCP are generally done at room temperature. The reaction time of 1-MCP is varied. Carnations treated with 1-MCP remained insensitive to ethylene for 12 - 15 days at 24°C (Sisler and Serek, 1997). Banana fruit treated with 1-MCP remained insensitive for 10 - 11 days after the control fruit treated with air displayed

increases in respiration and ethylene production (Golding et al., 1998). Ripening of apples can be inhibited for as long as 25 - 35 days more than the control fruit at room temperature by a single exposure to 1-MCP (Fan et al., 1999).

It was originally thought that increased CO_2 levels acted in the same way as 1-MCP. De Wild et al. (1999), however, proved that CO_2 acts more as a non-competitive inhibitor in the preliminary stages of ethylene production, resulting in a synergistic effect when the two treatments were combined. We hypothesise that treatment of 'Fuerte' and 'Hass' avocados with 1-MCP alone or in combination with CA will extend shelf life, improve firmness and fruit quality.

Materials and Methods

Season 1

Experimental set up: 'Fuerte' avocado fruit were harvested and transported to the University of Stellenbosch by Summerfield exporters (harvest and packaging dates not known). Fruit size ranged between count 10 and count 14 (266 g - 450 g) and was intended for the local market. 'Hass' avocado fruit were harvested and transported to the University of Stellenbosch by Westfalia exporters (harvest and packaging dates not known). Fruit size was count 16 (236g - 265g) and was intended for the export market.

The fruit was immediately sorted on arrival ('Fuerte': 22nd June 2001, 'Hass': 4th September 2001) and all damaged fruit were discarded. The fruit were stored at 5.5°C for 18 days from arrival, simulating the approximate commercial shipping period from South Africa. Thereafter, temperatures were increased to 20°C until all fruit were eating ripe, to simulate a shelf life period in air. The treatments for 'Fuerte' were: regular atmosphere (RA) and CA.

The treatments for 'Hass' were: RA with 1-MCP (RA 1-MCP) and without 1-MCP (RA) and CA with 1-MCP (CA 1-MCP) and without 1-MCP (CA). The fruit were treated with 500 nl.l^{-1} 1-MCP on arrival for 24 hours at room temperature before being moved into RA and CA at 5.5°C.

Fruit were placed in 25 L buckets and connected to humidified RA or CA supplied via flow boards. Flow rates were about 450 $ml.min^{-1}$ during storage and shelf life. The atmosphere composition was checked regularly and maintained within 10% of the required concentrations using an O_2 / CO_2 analyser (PBI-Dansensor, Combi Check 9800-1, Ringsted, Denmark). The 'Fuerte' experiment was a randomised block design with two treatments each consisting of three replications with 25 fruit each. The 'Hass' experiment was a randomised block design with four treatments each consisting of four replications with 30 fruit each.

A representative set of 20 fruit was taken initially and evaluated for firmness prior to the fruit being put under atmosphere. Thereafter, five fruit per replication were removed for firmness evaluation after 18 days of storage. For the 'Fuerte' experiment during the shelf life period 14 fruit per replication and 'Hass' experiment 20 fruit per replication were removed for evaluation as they reached the eating ripe stage. This was assessed by gently squeezing the fruit by hand.

Maturity indices

Firmness. Readings were taken on opposite sides of the peeled fruit with a penetrometer (Southtrade fruit pressure tester, FT 327, Alphonsine, Italy) fitted with a 5 mm tip.

Moisture content. Moisture content was measured only initially when the fruit arrived. It was only done once as moisture content does not change much during the storage period and is used as a maturity index for harvest. Moisture content was determined by the method described by Swarts (1978). The fruit was cut in half and the pip removed. The fruit was grated at the cut surface and weighed. The sample was placed in a microwave on high for two minutes after which it was reweighed. The sample was replaced in the microwave for a further two minutes and reweighed, and the process repeated until a constant mass was achieved. The difference between the initial mass of the sample and the final mass of the sample as a percentage of the initial mass of the sample gave the moisture content of the fruit. This was done on three fruit. For each new two minute cycle a beaker of cold water was placed in the microwave with the fruit sample, to prevent burning of the sample.

Disorders. Fruit were evaluated when eating ripe during the shelf life period. Fruit were rated for external disorders: chilling injury, black cold, Dothiorella / Colletotrichum complex (D/C) and lenticel damage. The fruit were then cut in half and allowed to stand for 10 minutes so that any internal disorders could become visible. The fruit were rated for internal disorders: pulp spot, grey pulp and vascular browning. The decay which was rated was: stem-end rot, internal anthracnose and external anthracnose. The statistics for the disorders was calculated as a percentage of the total number of fruit per replication evaluated for disorders.

Respiration rate. CO_2 levels were measured with the use of an infra-red gas analyser (IRGA) (Infra-Red Gas Analyser, S151, Kingston, Ontario), which was connected to the out flow from each of the buckets. Three fruit during the 18 days storage and one fruit during the seven days shelf life were enclosed in 5 L buckets to measure respiration and ethylene production. Readings were taken approximately every third day during the storage time and every day during the shelf life period. The measurements on fruit treated with CA, were taken after the respective treatments were complete and the fruit stored in air. This was because the controlled atmospheres had CO_2 levels greater than 0.2% (or 2000 $\mu l.l^{-1}$), which is the upper limit of the IRGA.

Ethylene production rate. Gas samples were taken from the out flow of each bucket, except the CA, on every third day during the storage period, and daily for all treatments during the shelf life period. Samples were analysed by gas chromatograph (GC Series 3000, Varian 4290 integrator, Varian Instrument Group, Palo Alto, California).

Internal ethylene content (IEC). A partial vacuum was applied on individual fruit with the use of a glass vacuum container with a gas tight lid and a vacuum pump (Saltveit, 1982). Within the container the fruit was held in a flask filled with water with a septum at the point were the gas accumulates when the vacuum is applied. After the vacuum had been applied and released a sample of the extracted gas was taken with a gas tight syringe and evaluated using a gas chromatograph. An initial representative set of six fruit were evaluated on the arrival date. On removal after

18 days storage, one fruit per replication was evaluated, and another fruit per replication again after 7seven days shelf life at 20°C.

Ripening rates. As the fruit were removed from the shelf life period the number of fruit per treatment and days at 20°C until eating ripe were recorded.

Statistical Analysis. Analysis of variance (ANOVA) of the main effects and LSD values with a significance level of 5% were obtained using Statistical Analysis Systems (SAS). Presented data points are the means of the replications ±SE.

Season 2

Experimental set up: 'Fuerte' avocado fruit were harvested on the 17th of April 2002 and transported at 7°C to the University of Stellenbosch by Westfalia exporters. Fruit size was count 14 (266 - 305 g) and was intended for the export market. 'Hass' avocado fruit were harvested on the 9th of May 2002 and transported at 7°C to the University of Stellenbosch by Summerfield exporters. Fruit size was count 14 and was intended for the export market.

The fruit was immediately sorted on arrival ('Fuerte': 22nd April 2002, 'Hass': 14th May 2002) and all damaged fruit were discarded. The fruit for treatment with 1-MCP were treated on the day of arrival overnight for 16 hours at 7°C with 300 nl.l^{-1} 1-MCP. Thereafter, the fruit were stored at 7°C to make up 28 days storage from the date of harvest to simulate the commercial transport period from South Africa. From the date of arrival, CA treatment was imposed for 18 days of the 28 days at 7°C and thereafter the fruit was stored in air for the balance of the 28 days. The treatments were: RA with 1-MCP (RA 1-MCP) and without 1-MCP (RA) and CA with 1-MCP (CA 1-MCP) and without 1-MCP (CA). Thereafter, temperatures were increased to 20°C until all fruit were eating ripe to simulate a shelf life period in air.

Fruit were placed in 25 L buckets and connected to humidified RA or CA supplied via flow boards. Flow rates were about 450 ml.min^{-1} during storage and shelf life. The atmosphere composition was checked regularly and maintained within 10% of the required concentrations using an O_2 / CO_2 analyser (Dual gas analyser, ICA 15, Tyler

House, Tonbridge). The experiments were a randomised block design with four treatments each consisting of four replications with 30 fruit each.

A representative set of 20 fruit was taken initially and evaluated for firmness prior to the fruit being treated. Thereafter, five fruit per replication were removed for firmness evaluation after 28 days of storage. During the shelf life period 20 fruit per replication were removed for evaluation as they reached the eating ripe stage. This was assessed by gently squeezing the fruit by hand.

Maturity indices

For both experiments the fruit were evaluated for the same maturity indices as in season 1.

Statistical Analysis. Analysis of variance (ANOVA) of the main effects and LSD values with a significance level of 5% were obtained using Statistical Analysis Systems (SAS). Presented data points are the means of the four replications ±SE.

Results

Season 1

Expt 1: 'Fuerte'

At the start of the experiment the fruit had a mean moisture content of 67.7% and were therefore stored at 5.5°C (Hardy et al., undated).

Firmness. On arrival the fruit had a mean firmness of 9.3 kg. After 18 days storage at 5.5°C the fruit stored under CA had a firmness of 9.8 kg, which was not significantly firmer than the fruit stored under RA (6.7 kg) (Table 1).

Disorders. The internal disorders were generally more prominent in the fruit stored under RA (Table 2). Those fruit had significantly higher levels of pulp spot (45.1%), which were absent in the fruit stored under CA. There were no significant differences in the occurrence of grey pulp and vascular browning.

The decay disorders were far less prominent and were all higher in the fruit stored under CA (Table 2). There was, however, no significant difference between treatments for any of the disorders. External anthracnose was the most prominent of the decay disorders.

Respiration rate. The respiration rates of the fruit stored under RA were relatively constant while the fruit were held at 5.5°C (± 25 $mg.kg^{-1}.h^{-1}$) (Fig. 1). With the onset of the shelf life period and increase in temperature to 20°C all the fruit had a sharp increase in respiration rate of at least 110 $mg.kg^{-1}.h^{-1}$. The fruit stored under CA had maximum respiration rates on day 21 (156.2 $mg.kg^{-1}.h^{-1}$) while the fruit stored under RA had maximum respiration rate on day 20 (138.0 $mg.kg^{-1}.h^{-1}$). Thereafter there was a steady decrease in respiration rate.

Ethylene production rate. The fruit stored under RA had measurable ethylene production on day 19 with the onset of the shelf life period reaching maximum production rate on day 22 (5.5 $\mu l.kg^{-1}.h^{-1}$) but not reaching a distinct peak (Fig. 2). The fruit stored under CA had measurable ethylene production after a day at 20°C reaching maximum on day 21 (11.8 $\mu l.kg^{-1}.h^{-1}$). Thereafter production slowed to 3.6 $\mu l.kg^{-1}.h^{-1}$ at the end of the experiment.

Internal ethylene content (IEC). On arrival the fruit had a mean IEC of 0.09 $\mu l.l^{-1}$ (Fig. 3). After 18 days storage the fruit stored under RA had an increase in IEC to 0.8 $\mu l.l^{-1}$. This was not significantly higher than IEC of the fruit stored under CA, which had little change from the initial value. After a further seven days at 20°C the fruit stored under RA had IEC levels approximately 20 $\mu l.l^{-1}$ less than the fruit stored under CA, but the difference was insignificant (RA: 25.7 $\mu l.l^{-1}$ and CA: 42.9 $\mu l.l^{-1}$).

Ripening rates. RA stored fruit attained eating ripe maturity from two days at 20°C (day 20) and were all eating ripe within five days (day 23) (Fig. 4). Ripening of the CA stored fruit was delayed by approximately two days, and all fruit were ripe within seven days (day 25).

Expt 2: 'Hass'

At the start of the experiment the fruit had a mean moisture content of 68.3% and were therefore stored at 5.5°C (Hardy et al., undated).

Firmness. On arrival the fruit had a mean firmness of 11.0 kg (Table 3). After 18 days storage at 5.5°C the fruit stored under CA were significantly firmer (12.2 kg) than the fruit stored under RA (10.1 kg) and RA 1-MCP (10.7 kg). There was no significant difference in firmness between the fruit stored under CA and CA 1-MCP (11.5 kg).

Disorders. The fruit stored under RA and RA 1-MCP had a significantly higher percentage of sound fruit (91.3% and 85.0%, respectively) (Table 4). The fruit stored under CA 1-MCP had the significantly least sound fruit (54.0%). Although there were no significant differences between treatments, the internal disorders were generally more prominent in the fruit stored under CA 1-MCP.

Fruit stored under RA and RA 1-MCP had the significantly lowest percentage of stem-end rot (3.8% and 5%, respectively), while the fruit stored under CA and CA 1-MCP had more than 20% infection (Table 4). Internal anthracnose was significantly highest in the fruit stored under CA 1-MCP (27.5%) while the fruit stored under RA had significantly less infected fruit (1.3%) than the fruit stored under CA and CA 1-MCP. There was no significant difference between any of the treatments for external anthracnose and it occurred at low levels. The remaining disorders for which the fruit were evaluated did not occur.

Respiration rate. There was a relatively constant respiration rate while the fruit were held at 5.5°C (± 10 $mg.kg^{-1}.h^{-1}$) (Fig. 5). With the onset of the shelf life period and subsequent increase in temperature to 20°C all the fruit had a sharp increase in respiration of at least 40 $mg.kg^{-1}.h^{-1}$. The fruit stored under RA had maximum respiration rate the earliest, on day 19 (86.8 $mg.kg^{-1}.h^{-1}$). The respiration rate of the fruit stored under CA, RA 1-MCP and CA 1-MCP continued to increase during the shelf life period and did not display a distinct peak before the end of the experiment.

Ethylene production rate. Ethylene production was first measurable with the onset of the shelf life period (Fig. 6). The fruit stored under RA had a relatively distinct peak in ethylene production (40.7 $ul.kg^{-1}.h^{-1}$), followed by a decline towards the end of the experiment. This was not the case for the fruit stored under RA 1-MCP and CA 1-MCP, which continued to increase production rate without reaching maximum production before the end of the experiment. CA 1-MCP had the highest ethylene production rate (74.6 $ul.kg^{-1}.h^{-1}$) on the final day of the experiment. The next highest was RA 1-MCP, also on the final day (58.1 $ul.kg^{-1}.h^{-1}$).

Internal ethylene content (IEC). On arrival the fruit had a mean IEC of 0.2 $\mu l.l^{-1}$ (Fig. 7). After 18 days storage there was no significant difference in IEC between treatments. The fruit stored under RA 1-MCP had the highest IEC (1.4 $\mu l.l^{-1}$). After the shelf life period at 20°C the significantly lowest IEC was found in the fruit stored under RA (107.9 $\mu l.l^{-1}$). The remaining treatments had no significant difference (206 - 272 $\mu l.l^{-1}$).

Ripening rates. All the treatments had eating ripe fruit after three days at 20°C (Fig. 8). The fruit stored under RA were all eating ripe within five days (day 23). The remaining treatments took five days longer before all fruit were eating ripe and the fruit stored under CA 1-MCP had the distinctly slowest ripening rate.

Season 2:

Expt 1: 'Fuerte'

At the start of the experiment the fruit had a mean moisture content of 76.9% and were therefore stored at 7°C (Hardy et al., undated).

Firmness. Only the fruit stored under RA had extreme softening, from an initial mean firmness of 9.3 kg to 1.5 kg after 18 days storage (Table 5). The fruit stored under CA and CA 1-MCP were significantly firmest (9.8 kg and 9.9 kg, respectively). The RA 1-MCP treated fruit were softer (9.1 kg), but still significantly firmer than the fruit stored under RA.

Disorders. The fruit stored under RA 1-MCP and CA 1-MCP had a significantly higher percentage of sound fruit (51.3 and 55.0%, respectively) than the fruit stored under RA (28.8%) (Table 6). Chilling injury was the only external disorder, occurring only in the fruit stored under RA (22.5%).

The internal disorders were all most prominent in the fruit stored under RA (Table 6). Only the fruit stored under RA had pulp spot (51.3%). The same fruit had a significantly higher occurrence of grey pulp (6.3%) than the fruit stored under CA, which were not affected. There was no significant treatment difference in the occurrence of vascular browning, although those fruit stored under RA 1-MCP and CA 1-MCP appeared less affected.

Stem-end rot was the most prominent of the decay disorders and the fruit stored under CA had significantly higher occurrence of the disorder (46.3%) than the fruit stored under RA (11.3%) (Table 6). Internal anthracnose occurrence was no higher than 2.5% and there were no significant differences between treatments. There were also no significant treatment differences in the occurrence of external anthracnose, although levels were higher than in the internal anthracnose.

Respiration rate. During the storage time at 7°C all the fruit had a relatively stable respiration rate (± 20 $mg.kg^{-1}.h^{-1}$) (Fig. 9). With the onset of the shelf life period and subsequent increase in temperature to 20°C all the treatments had sharp increases in respiration rate. The fruit stored under RA reached maximum respiration rate on day 29 (173.6 $mg.kg^{-1}.h^{-1}$) and the fruit stored under CA on day 31 (190.6 $mg.kg^{-1}.h^{-1}$). Thereafter those treatments had a slight decrease and increase again in respiration rate due to decay and overripeness. The fruit stored under RA 1-MCP and CA 1-MCP did not have a distinct peak in respiration rate but continued to increase through the shelf life period and had very similar respiration rates on the final day of the experiment (± 150 $mg.kg^{-1}.h^{-1}$).

Ethylene production rate. The first signs of ethylene production was on day 19 in the fruit stored under RA (Fig. 10). All the treatments had a sharp increase in ethylene production rate with the onset of the shelf life period. None of the treatments reached a peak in ethylene production, reaching their highest rates on the final day of the

experiment. The fruit stored under RA and CA 1-MCP reached similar levels on the final day (± 35.6 $ul.kg^{-1}.h^{-1}$). The fruit stored under RA 1-MCP had the lowest levels on day 37 (± 17.5 $ul.kg^{-1}.h^{-1}$).

Internal ethylene content (IEC). All the treatments except the fruit stored under RA had a decrease in IEC after 28 days storage from the initial mean of 0.22 $\mu l.l^{-1}$ (Fig. 11). Thus after storage the fruit stored under RA had the highest IEC. After nine days of shelf life at 20°C the fruit stored under CA had the significantly highest IEC (147.9 $\mu l.l^{-1}$). There was no significant difference between the remaining treatments.

Ripening rates. The fruit stored under RA ripened distinctly faster than the other treatments (Fig. 12). The fruit stored under RA 1-MCP and CA 1-MCP ripened the slowest with all fruit eating ripe within 15 and 13 days at 20°C, respectively.

Expt 2: 'Hass'

At the start of the experiment the fruit had a mean moisture content of 77.2% and were therefore stored at 7°C (Hardy et al., undated).

Firmness. The mean firmness of the fruit on arrival was 11.5 kg (Table 7). The fruit stored under CA 1-MCP had little change in firmness from the initial firmness value (11.9 kg) being significantly firmer than both the fruit stored under RA (7.8 kg) and RA 1-MCP (10.6 kg). The fruit stored under RA were significantly softest.

Disorders. There was no significant difference between treatments in the percentage of sound fruit and values ranged between 71.3 - 86.3% (Table 8). There was no significant difference between treatments for the internal disorders. Only the fruit stored under RA 1-MCP had grey pulp (1.3%) while there was 12.5% vascular browning of the fruit stored under RA 1-MCP and CA 1-MCP.

Of the decay disorders, only external anthracnose had significant differences (Table 8). The fruit stored under CA 1-MCP (5.0%) had significantly higher levels

than the fruit stored under RA (0%). Stem-end rot was more prominent in the CA and CA 1-MCP treated fruit although no significantly so.

Respiration rate. During the storage time at 7°C the fruit had a relatively constant respiration rate (± 10 $mg.kg^{-1}.h^{-1}$) (Fig. 13). With the onset of the shelf life period at 20°C all the treatments had a sharp increase in respiration rate. The fruit stored under RA had maximum respiration rate on day 29 (183.4 $mg.kg^{-1}.h^{-1}$) while the fruit stored under RA 1-MCP reached maximum respiration rate on day 31 (169.6 $mg.kg^{-1}.h^{-1}$). The fruit stored under CA had an increase in respiration rate with the onset of the shelf life period, and had a second increase on day 33, reaching maximum respiration rate on day 36 (131.1 $mg.kg^{-1}.h^{-1}$). The fruit stored under CA 1-MCP reached maximum respiration rate on day 32 (95.3 $mg.kg^{-1}.h^{-1}$).

Ethylene production rate. The fruit stored under RA and RA 1-MCP reacted immediately with the onset of the shelf life period, having an increase in ethylene production and reaching maximum on day 32 (Fig. 14). The subsequent increases were due to decay and overripe fruit. The fruit stored under CA 1-MCP had first signs of ethylene production on day 31, reaching maximum on day 32 (38.4 $ul.kg^{-1}.h^{-1}$) and displayed a subsequent decrease in production. The fruit stored under CA had first signs of ethylene production on day 33 and did not have a distinct peak in production by the end of the experiment, reaching 99.3 $ul.kg^{-1}.h^{-1}$.

Internal ethylene content (IEC). The fruit generally had an increase in IEC from the start of the experiment until after the shelf life period (Fig. 15). Only the fruit stored under RA had a large increase in IEC after 28 days storage, reaching 7.6 $\mu l.l^{-1}$. After five days at 20°C (day 33) the fruit stored under RA 1-MCP had the significantly highest IEC (375.1 $\mu l.l^{-1}$) while the remaining treatments had no significant difference and ranged between 57.3 $\mu l.l^{-1}$ and 108.3 $\mu l.l^{-1}$. After the shelf life period the fruit stored under CA and CA 1-MCP had significantly highest IEC (175.6 $\mu l.l^{-1}$ and 170.4 $\mu l.l^{-1}$, respectively).

Ripening rates. The fruit stored under RA had a distinctly faster ripening pattern, with all fruit eating ripe within six days (day 34) (Fig. 16). The fruit stored CA and RA 1-MCP had similar ripening patterns, although the fruit stored under RA 1-MCP

took a day longer until fully eating ripe. The fruit stored under CA 1-MCP had the slowest ripening pattern.

Discussion and Conclusion

Firmness. It was apparent from both seasons in our experiments that those fruit stored under CA had firmness levels which ranged from 2 - 8 kg firmer than the fruit stored under RA, after the storage time. Polygalacturonase (PG) and pectinesterase (PE) are enzymes involved in the hydrolysis of cell wall pectin (Dong et al., 2001a). Awad and Young (1979) found that cellulase and PG activity in 'Fuerte' avocados increased during fruit ripening while PE activity decreased. Bower and Cutting (1988) were in agreement with this and further suggested that cellulase appeared to be responsible for the early stages of avocado fruit softening and PG for final fruit softening. Furthermore it has been found that PG is ethylene regulated (Sitrit and Bennett, 1998). This, coupled with the inhibitory effect that increased CO_2 levels have on the enzymes involved in ethylene production, explains partly why there is reduced loss of firmness in the CA treated fruit.

The extreme levels of CO_2 in the CA treatments results in a build up of CO_2 within the fruit via diffusion. These increased levels of CO_2 prevent extensive loss of sugars and overripening and thus delay fruit softening (Salisbury and Ross, 1991). Lange and Kader (1997) found that storage of 'Hass' avocados in CO_2 enriched atmospheres (20% CO_2 and 17% O_2 or 40% CO_2 and 13% O_2) or in air softened similarly during storage after five days at 20°C. They went further to say that five days at 20°C was not long enough for softening to occur in preclimacteric 'Hass' avocados. Knee (as reported by Kader, 1986) found that apples stored under CA had flesh softening rates half of maximal at 2.5 - 4.0% O_2. Similar results were found by Bender (1989) in an experiment with 'Gala' apples, where fruit treated with 1.1% O_2 and 3.2% CO_2 had flesh firmness levels more than 20 $N.cm^{-2}$ firmer than the control fruit.

During season one of our experiments, there was no significant difference between the fruit stored under RA 1-MCP and the fruit stored under RA alone. However, during season 2, the fruit stored under RA 1-MCP were significantly firmer than the fruit stored under RA alone. The prevention of ethylene action by 1-MCP means that

1-MCP treated fruit should be firmer than untreated fruit, as ethylene is directly involved in PG regulation and thus fruit softening. This has been found by 1-MCP treatment of apples (Rupasinghe et al., 2000a; Rupasinghe et al., 2000b), 'Red Rosa' plums (Dong et al., 2001b), 'Flavortop' nectarine (Dong et al., 2001a), 'Hass' (White et al., 2001) and 'Quintal' avocados (Kluge et al., 2002). Nazir et al. (2001) suggested that the effectiveness of 1-MCP on 'Redchief Delicious' apples at elevated temperatures (≥ 5°C) can be improved with frequent applications.

It was further found by De Wild et al. (1999) that the increased CO_2 levels and 1-MCP treatments had a synergistic effect as they act at different points in the ethylene production process. This combined effect was seen in our experiments during season two, where the fruit treated with CA and 1-MCP (CA 1-MCP) were the firmest treatment after storage although only significantly firmer than the fruit treated with 1-MCP alone (RA 1-MCP) and not the fruit stored under CA. The same combined effect of the two treatments was not apparent during season one.

Disorders. 'Fuerte' avocados, which are known to be susceptible to pulp spot (a chilling injury disorder), gained large benefits during both seasons from treatment with CA and 1-MCP when compared to the fruit stored under RA alone, as the disorder was completely prevented. The same was apparent for external chilling injury. With regard to the remaining disorders results were varied. In contrast 'Hass' avocados are known to be far less susceptible to disorders than some other cultivars. This is most clearly seen as the fruit stored under RA alone had of the lowest incidence of each mentioned disorder and had the highest percentage of sound fruit during both seasons.

The chilling sensitivity of 'Fuerte' and 'Hass' avocado is dependant on the stage of the ethylene climacteric (Donkin, 1995). The fruit are less sensitive at the climacteric rise than at the climacteric peak and least sensitive post-climacteric (Donkin, 1995). Due to its role in chilling injury White et al. (2001) recommend that avocados are stored under low ethylene conditions. Chilling injury symptoms include: blackening and pitting of the exocarp, grey-brown discolouration of the mesocarp and uneven ripening (Couey, 1982).

The oxidation of o-diphenols to o-quinones by the enzyme polyphenoloxidase (PPO) results in these browning reactions of the mesocarp (Bower and Cutting, 1988). CA (2% O_2 and 10% CO_2) stored 'Fuerte' avocado fruit had the lowest PPO activity during storage (Truter et al., 1992). PPO activity increased during softening to reach the highest levels when compared to the control and CO_2 shock (25% CO_2 and O_2 decreasing to 1% after three days) treated fruit. However, the CA and CO_2 shock treated fruit generally had the fewest internal disorders. This is due to a change in PPO activity just before softening (Cutting et al., 1990) and probably the stage that ripening takes place (Truter et al., 1992). Therefore during this process the inactivation of PPO results in a lower activity where browning has occurred as opposed to CA where no browning has taken place (Truter et al., 1992).

Flesh greying and internal chilling injury of 'Hass' avocados was almost completely restricted after seven weeks storage by treatment with 1-MCP while the control fruit had approximately 90% affected (White et al., 2001). They also suggested that lower concentrations of 1-MCP ($\leq$ 250 $nl.l^{-1}$) are optimal otherwise fruit will tend to rot before they ripen.

Respiration rate. Temperature has the primary influence on the respiration rate of fruit (Blanke, 1991). This would explain the distinct rises in respiration rate for all the fruit of our experiments on exposure to 20°C during the shelf life period, regardless of treatment. This relationship between temperature and ripening is expressed as the temperature coefficient (Q_{10}), which describes the increase in respiration for a 10°C rise in temperature (Kader, 2002). For most non-chilling sensitive commodities an increase of 10°C above the optimum storage temperature will result in a two to three fold increase in respiration and, thus, deterioration.

The rise in respiration during ripening of certain fruit without an increase in temperature, was named the respiratory climacteric by Kidd and West (as reported by Blanke, 1991). For avocado fruit this is facilitated by detachment from the tree (Blanke, 1991). For the 'Fuerte' avocados in our experiments, treatment of the fruit with CA successfully delayed the onset of the climacteric by one to two days,

regardless of the increase in temperature. These peaks in respiration rate were, however, not decreased in the fruit stored under CA. On the other hand, in the 'Hass' avocados, treatment with CA delayed the onset of the climacteric and also successfully decreased the respiration rate of the fruit when compared to the fruit stored under RA. This is possibly purely a cultivar or seasonal influence.

The use of CA in fruit storage reduces the gradient of CO_2 from the fruit to the ambient atmosphere and therefore causes an accumulation of CO_2 within the fruit (Blanke, 1991). This, according to Blanke (1991), causes a slowing down in activity of malate decarboxylase and of some respiratory enzymes, thus slowing down or retarding the respiratory climacteric. Furthermore, the inhibitory effect of CO_2 results from a decreased succinate dehydrogenase activity causing an initial irreversible accumulation of succinate and so, according to Hulme, suppressing apple respiration (as reported by Blanke, 1991). This further agrees with Young et al. (1962), who found that levels of CO_2 between 5 - 10% for 21 days depressed respiration of avocado fruit and delayed the climacteric rise in respiration. 'Bartlett' pears treated with air, air and 5% CO_2, air and 10% CO_2 and air and 20% CO_2 after four days at 20°C, had respiration rates of 35, 27, 20 and 15 ml $O_2.kg^{-1}.h^{-1}$, respectively (Ong, as reported by Kader, 1989). Thus, the decrease in respiration rates and the delay of the onset of the climacteric were proportional to the decrease in O_2 concentration and increase in CO_2 concentration.

The fruit treated with RA 1-MCP had respiration rates lower than the fruit stored under RA as the fruit were transferred to the shelf life period at 20°C during the 'Hass' experiment in season one and the 'Fuerte' experiment in season two. A few days later, however, the trend was reversed, possibly as the effects of the 1-MCP wore off and respiration rates of the RA 1-MCP treated fruit continued to rise while the fruit stored under RA respired slower. De Wild et al. (1999) found that the average respiration rate of pear fruit treated with 280 $nl.l^{-1}$ 1-MCP and held in air for three to five days was significantly lower than the control. Similarly, Golding et al. (1998) found that bananas treated with 1-MCP always had respiration rates lower than the control fruit.

De Wild et al. (1999) discussed the combined effect of treatment of CA and 280 $nl.l^{-1}$ 1-MCP on pears. They found a synergistic effect by this treatment as respiration rate slowed from 12.4 to 10.3 $nmol.kg^{-1}.s^{-1}$ with the addition of the 1-MCP before treatment of the fruit with CA. The same was not as apparent during both seasons in our experiments, as the fruit treated with both CA and 1-MCP (CA 1-MCP) respired at a similar rate to either the RA 1-MCP or CA treated fruit.

Ethylene production rate. The ethylene production rate of the fruit stored under CA was far lower than the fruit stored under RA with the onset of the shelf life period. From that point as the ethylene production rate of the fruit stored under RA decreased, that of the fruit stored under CA generally continued to increase without reaching a maximum before the end of the experiment, possibly as the effects of the CA wore off. According to Kader (1986) elevated CO_2 levels can reduce, promote or have no effect on ethylene production depending on the commodity and CO_2 concentration. O_2 levels below 8% decrease ethylene production and the sensitivity to ethylene in fresh fruit and vegetables (Kader, 1986). Burg and Burg (1967) also demonstrated that O_2 is needed for the production and action of ethylene.

During both seasons of our experiments the fruit treated with RA 1-MCP followed a similar ethylene production pattern to the fruit stored under RA. Untreated 'Gulfruby' plums and 'Beauty' plums show a typical climacteric. When treated with 1-MCP ripening and ethylene production was delayed for several days (Abdi et al., 1998). This was also apparent in the suppressed-climacteric phenotype: 'Shiro' plums and 'Rubyred' plums. Similar results were found on 'Hass' avocados which treated with 30 $nl.l^{-1}$ 1-MCP or higher (Feng et al., 2000). A subsequent treatment with 300 $\mu l.l^{-1}$ ethylene for 24 hours had no effect on the ripening of the avocados and the peak in ethylene production was delayed by 12 - 13 days when stored at 22°C. The results were the same when pears were treated with 280 $nl.l^{-1}$ 1-MCP (De Wild et al., 1999). There was a significant drop in ethylene production of approximately 15 $pmol.kg^{-1}.s^{-1}$ compared to the control. A possible reason why a clear delay in the climacteric was not observed in our experiments is because the fruit reached the climacteric during storage and the delay was not observed due to the low storage temperatures. The subsequent increase in ethylene production rate with the onset of the shelf life period is possibly purely a reaction to the increased temperature.

Notable is that for both seasons the 'Hass' avocados treated with RA 1-MCP reached higher ethylene production rates than the fruit stored under RA. Golding et al. (1998) found similar large increases in ethylene production of banana fruit treated with 1-MCP when compared to the control fruit.

De Wild et al. (1999) went on to discuss the combined effect of treatment with CA and 280 $nl.l^{-1}$ 1-MCP on pears. The ethylene production was significantly less in the combined treated fruit than the fruit treated with only CA and only 1-MCP. When considering the 'Hass' avocados during season one of our experiments the CA treated fruit reached a peak and tapered off while the RA 1-MCP treated fruit did not reach a distinct peak before the end of the experiment (Fig. 6). The ethylene production rate of the CA 1-MCP treated fruit was far more exaggerated than the RA 1-MCP treated fruit, still increasing at the end of the experiment and at that point more than 15 $ul.kg^{-1}.h^{-1}$ higher than any of the other treatments. Furthermore, the increase in ethylene production rate started two days later than any of the other treated fruit.

Internal ethylene content (IEC) and ripening rates. Ethylene is a hormone directly involved in fruit ripening (Lelièvre et al., 1997). During season two of our experiments, the 'Fuerte' avocados treated with CA, 1-MCP or both, had a decrease in IEC from the initial level until after the storage time. Apart from those fruit, the general trend was an increase in IEC as the experiment progressed, regardless of treatment. A second pattern which developed was that in general after the storage period the fruit stored under RA had higher IEC than any other treatment. In all cases after the shelf life period, however, the fruit stored under RA had the lowest IEC.

The fruit stored under RA therefore accumulated higher levels of internal ethylene earlier than the other treatments and so ripened faster than the other treatments. This was expected because, as stated earlier, high CO_2 levels delay softening and ripening. Feng et al. (2000) found significant delays in fruit ripening following 1-MCP treatment. There was also a combined effect of treatment with both CA and 1-MCP, during our experiments, as the fruit stored under CA 1-MCP generally had a slower ripening pattern than the CA or 1-MCP treatments.

When considering the proposed hypothesis, the improvement of fruit firmness and shelf life extension of 'Fuerte' and 'Hass' avocados were met when the fruit were stored under CA, RA 1-MCP and CA 1-MCP compared to storage under RA alone. Most importantly, those treatments were also able to prevent development of pulp spot disorder in 'Fuerte' avocados. There were, however, varying degrees of success with regard to the other disorders on those fruit. The 'Hass' avocados which are generally less prone to internal disorders, did not benefit to the same degree as the 'Fuerte' with CA and 1-MCP treatments.

1-MCP has been shown for a number of years to be an important tool for the control of ethylene responses over extended periods of time. It is, however, still not commercially available, and much still needs to be learned to what extent it can control ethylene responses in plants. Furthermore treatment of the fruit with 1-MCP alone very often had within fruit uneven ripening where the proximal half of the fruit was still relatively firm while the distal half was eating ripe.

Thus, to improve firmness, extend shelf life and improve internal quality of some avocados, treatment with RA 1-MCP alone would be the best option in terms of application practicality and cost effectiveness. However, for a cultivar such as 'Hass' which is relatively free of disorders, the benefit of improved firmness and shelf life from treatment with 1-MCP must be weighed up against the increased level of disorders which the treatment will cause.

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Table 1

Firmness (kg) of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or controlled atmosphere (CA).

	Firmness (kg)		
Initial	9.3		
RA		6.7	ns[z]
CA		9.8	
LSD		*3.6748*	

[z] Means separation within columns using least significant difference (0.05)

Table 2

Internal and external disorders (%) of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or controlled atmosphere (CA), followed by 20°C in air until eating ripe.

	Pulp spot (%)	Grey pulp (%)	Vascular browning (%)	Stem-end rot (%)	Anthracnose (%)	
					Internal	External
RA	45.1 a[z]	17.7 ns	7.8 ns	2.0 ns	3.9 ns	3.9 ns
CA	0.0 b	10.3	18.0	7.7	5.1	10.3
LSD	*14.406*	*17.076*	*28.992*	*13.475*	*8.9591*	*13.004*

[z] Means separation within columns using least significant differences (0.05)

Table 3

Firmness (kg) of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA).

	Firmness (kg)	
Initial	11.0	
RA	10.1	b[z]
RA 1-MCP[y]	10.7	b
CA	12.2	a
CA 1-MCP	11.5	ab
LSD	*1.3706*	

[z] Means separation within columns using least significant differences (0.05)

[y] *500 nl.l^{-1} 1-MCP for 24 hours on arrival*

Table 4

Internal and external disorders (%) of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA), followed by 20°C in air until eating ripe.

	Sound fruit (%)	Grey pulp (%)	Vascular browning (%)	Stem-end rot (%)	Anthracnose (%)	
					Internal	External
RA	91.3 a[z]	1.3 ns	3.8 ns	3.8 b	1.3 c	0.0 ns
RA 1-MCP[y]	85.0 a	2.5	3.8	5.0 b	7.5 bc	0.0
CA	71.5 b	2.5	5.0	21.0 a	12.4 b	2.5
CA 1-MCP	54.0 c	5.0	16.1	28.4 a	27.5 a	2.5
LSD	*13.179*	*7.291*	*12.601*	*12.494*	*10.779*	*5.447*

[z] Means separation within columns using least significant differences (0.05)

[y] *500 nl.l^{-1} 1-MCP for 24 hours on arrival*

Table 5

Firmness (kg) of 'Fuerte' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days.

	Firmness (kg)	
Initial	9.3	
RA	1.5	c[z]
RA 1-MCP[y]	9.1	b
CA	9.8	a
CA 1-MCP	9.9	a
LSD	*0.5402*	

[z] Means separation within columns using least significant differences (0.05)

[y] *300 nl.l^{-1} 1-MCP for 16 hours on arrival*

Table 6

Internal and external disorders (%) of 'Fuerte' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days, followed by 20°C in air until eating ripe.

	Sound fruit (%)	Chilling injury (%)	Pulp spot (%)	Grey pulp (%)	Vascular browning (%)	Stem-end rot (%)	Anthracnose Internal (%)	Anthracnose External (%)
RA	28.8 b[z]	22.5 a	51.3 a	6.3 a	41.3 ns	11.3 b	2.5 ns	11.3 ns
RA 1-MCP[y]	51.3 a	0.0 b	0.0 b	1.3 ab	26.3	36.3 ab	2.5	17.5
CA	43.8 ab	0.0 b	0.0 b	0.0 b	40.0	46.3 a	1.3	11.3
CA 1-MCP	55.0 a	0.0 b	0.0 b	5.0 ab	21.3	32.5 ab	2.5	7.5
LSD	*21.589*	*10.19*	*12.331*	*6.09*	*21.816*	*25.67*	*4.3063*	*14.325*

[z] Means separation within columns using least significant differences (0.05)

[y] *300 nl.l^{-1} 1-MCP for 16 hours on arrival*

Table 7

Firmness (kg) of 'Hass' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days.

	Firmness (kg)	
Initial	11.5	
RA	7.8	c[z]
RA 1-MCP[y]	10.6	b
CA	11.4	ab
CA 1-MCP	11.9	a
LSD	*1.2793*	

[z] Means separation within columns using least significant differences (0.05)

[y] *300 nl.l^{-1} 1-MCP for 16 hours on arrival*

Table 8

Internal and external disorders (%) of 'Hass' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days followed by 20°C in air until eating ripe.

	Sound fruit (%)	Grey pulp (%)	Vascular browning (%)	Stem-end rot (%)	Anthracnose (%)	
					Internal	External
RA	86.3 ns[z]	0.0 ns	7.5 ns	7.5 ns	3.8 ns	0.0 b
RA 1-MCP[y]	80.0	1.3	12.5	13.8	5.0	1.3 ab
CA	77.5	0.0	10.0	20.0	3.8	1.3 ab
CA 1-MCP	71.3	0.0	12.5	21.3	7.5	5.0 a
LSD	*17.297*	*1.9258*	*12.381*	*17.859*	*7.8621*	*4.1602*

[z] Means separation within columns using least significant differences (0.05)

[y] *300 nl.l^{-1} 1-MCP for 16 hours on arrival*

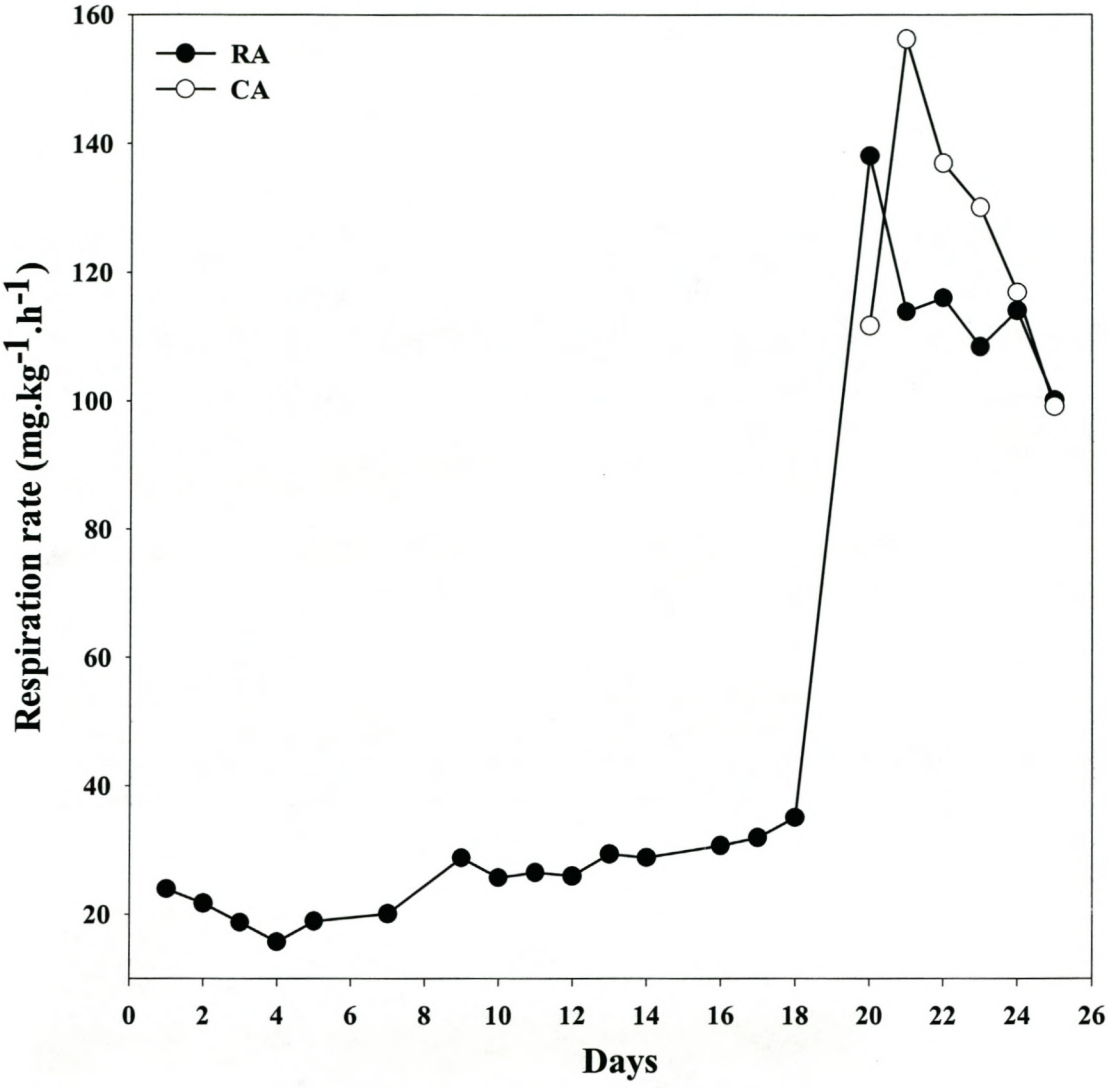

Fig. 1. Respiration rate (CO_2 evolved in $mg.kg^{-1}.h^{-1}$) of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or controlled atmosphere (CA), followed by 20°C in air until eating ripe. An LSD value was calculated for the shelf life period (*LSD = 30.648*).

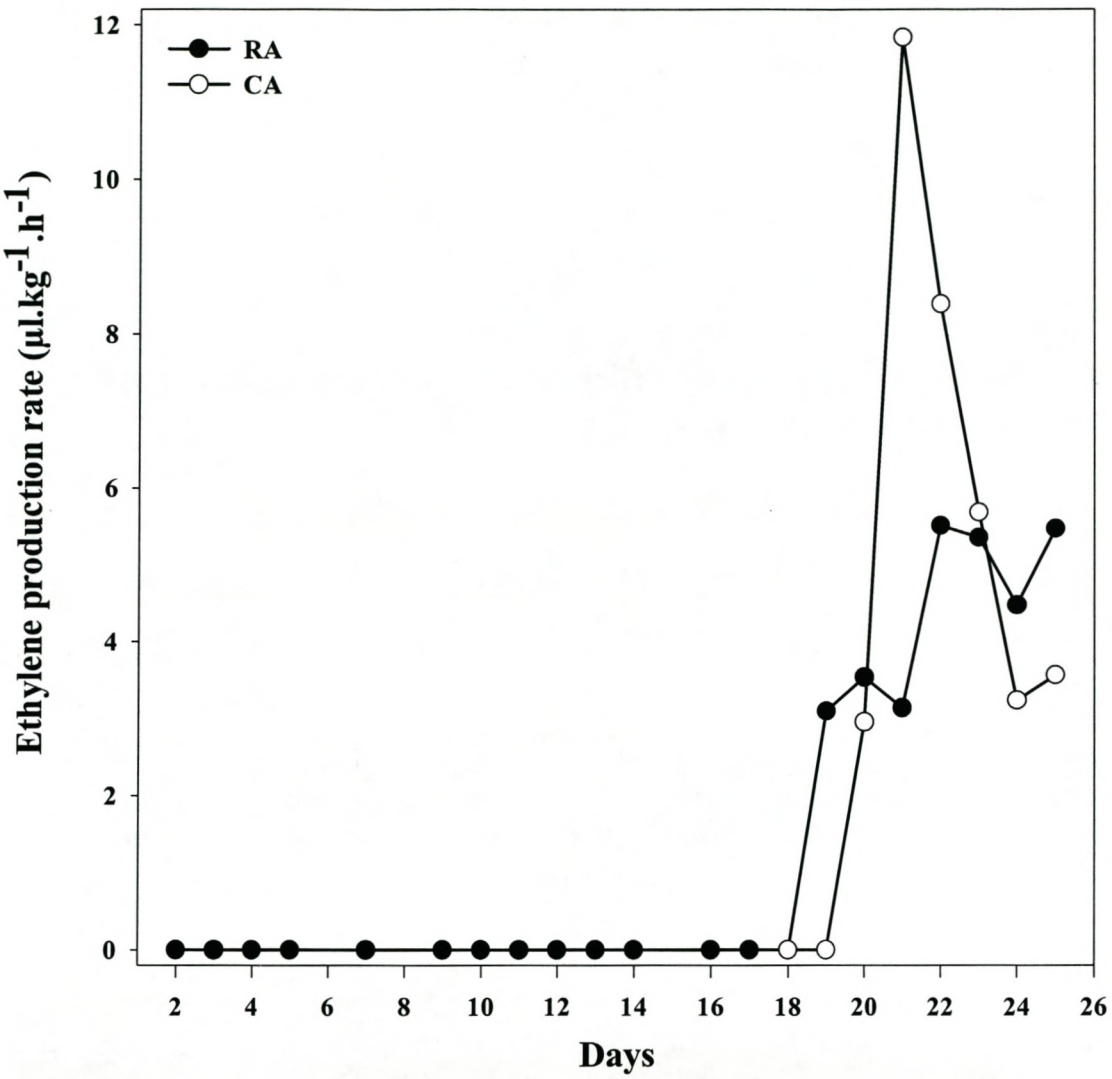

Fig. 2. Ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or controlled atmosphere (CA), followed by 20°C in air until eating ripe. An LSD value was calculated for the shelf life period (*LSD = 5.9118*).

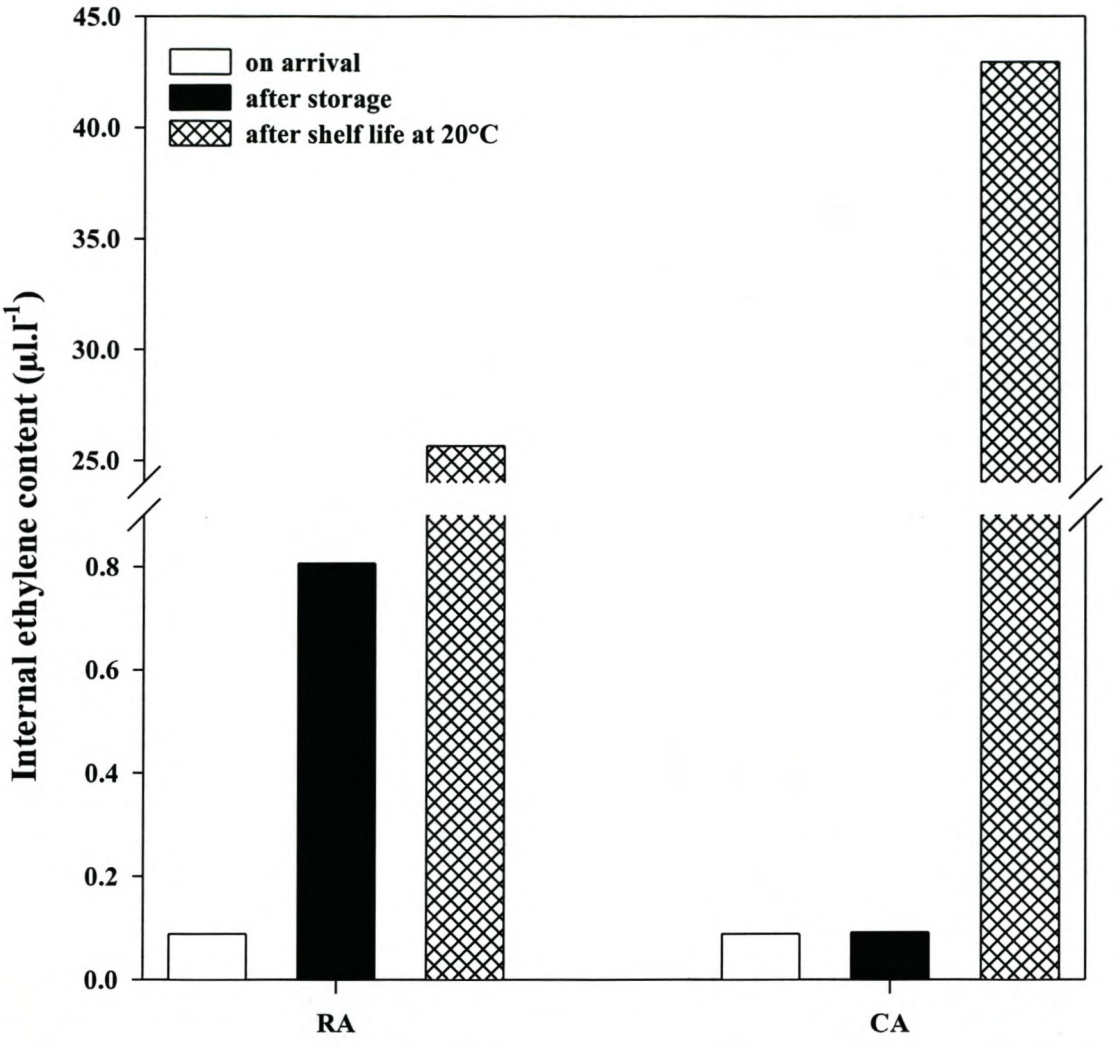

Fig. 3. Internal ethylene content ($\mu l.l^{-1}$) of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or controlled atmosphere (CA), followed by 20°C in air until eating ripe. Measurements were taken initially on arrival, after 18 days storage (*LSD = 0.8119*) and after seven days of shelf life at 20°C (*LSD = 29.66*).

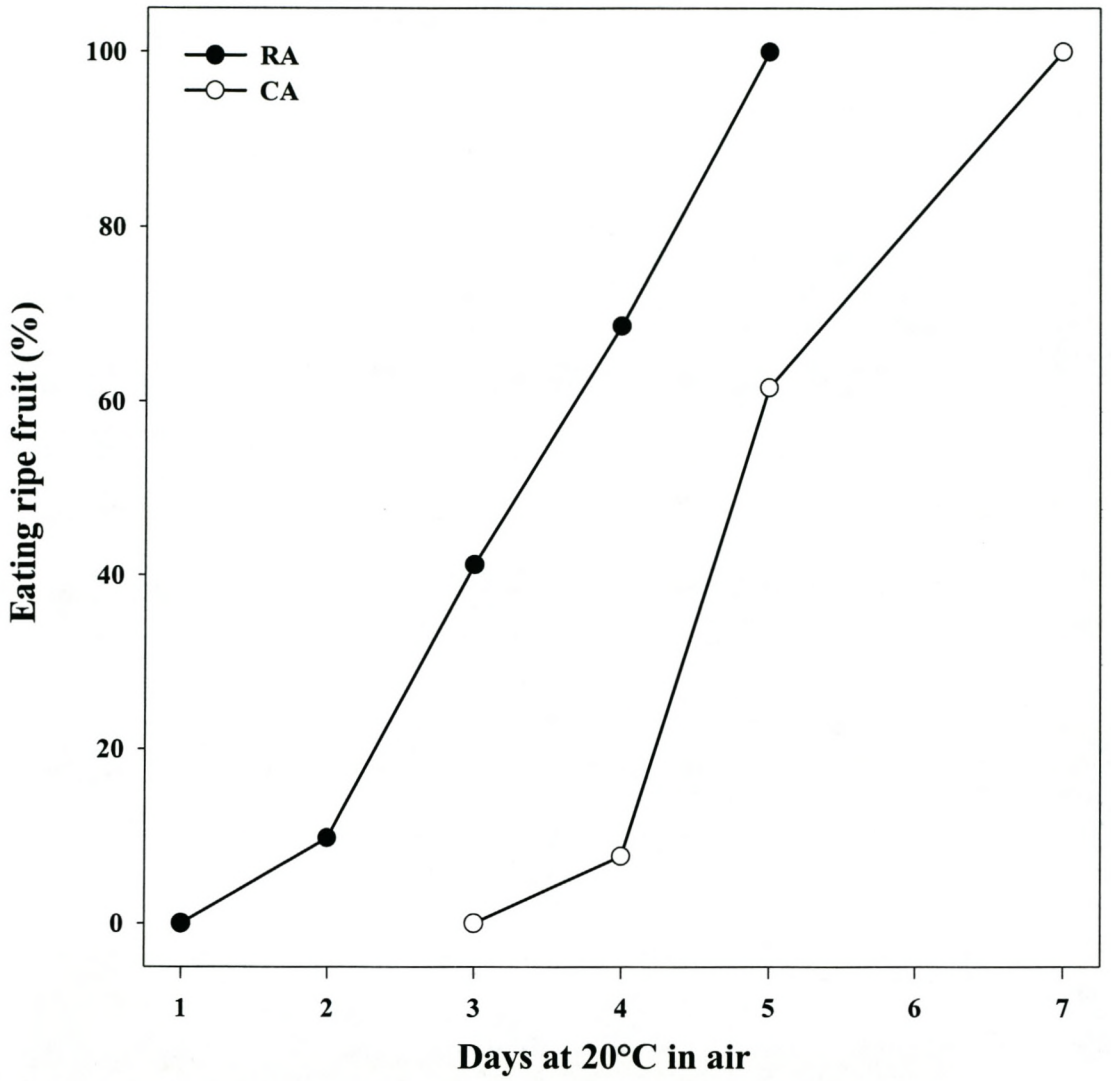

Fig. 4. Ripening rates of 'Fuerte' avocado fruit stored at 5.5°C for 18 days under regular atmosphere (RA) or controlled atmosphere (CA), followed by 20°C in air until eating ripe and the percentages recorded.

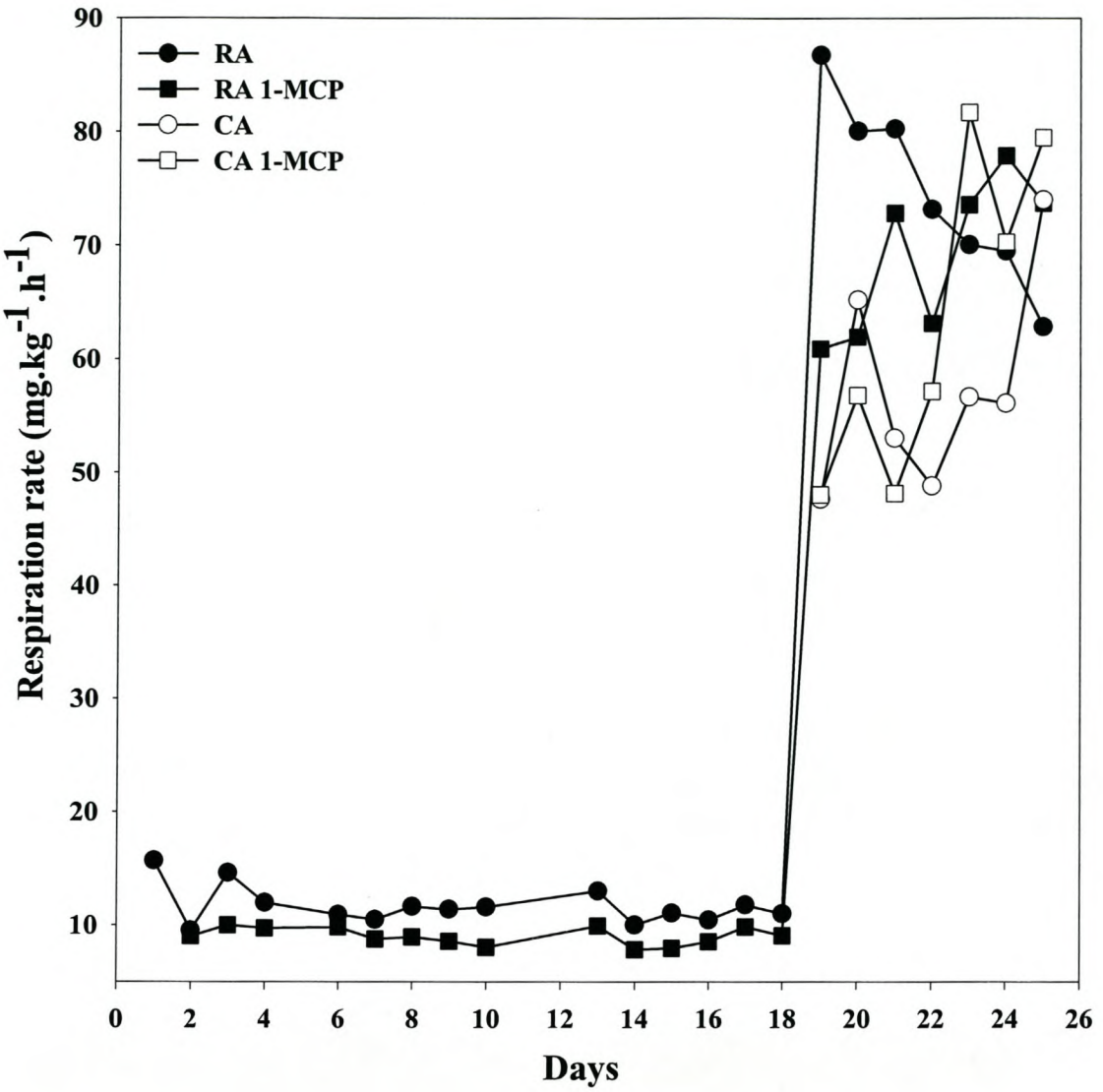

Fig. 5. Respiration rate (CO_2 evolved in $mg.kg^{-1}.h^{-1}$) of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA), followed by 20°C in air until eating ripe. LSD values were calculated after 18 days storage (*LSD = 2.1761*) and after the shelf life period (*LSD = 27.609*). *1-MCP treatment involved a 24 hour exposure at 500 nl.l^{-1} at room temperature immediately prior to cold storage.*

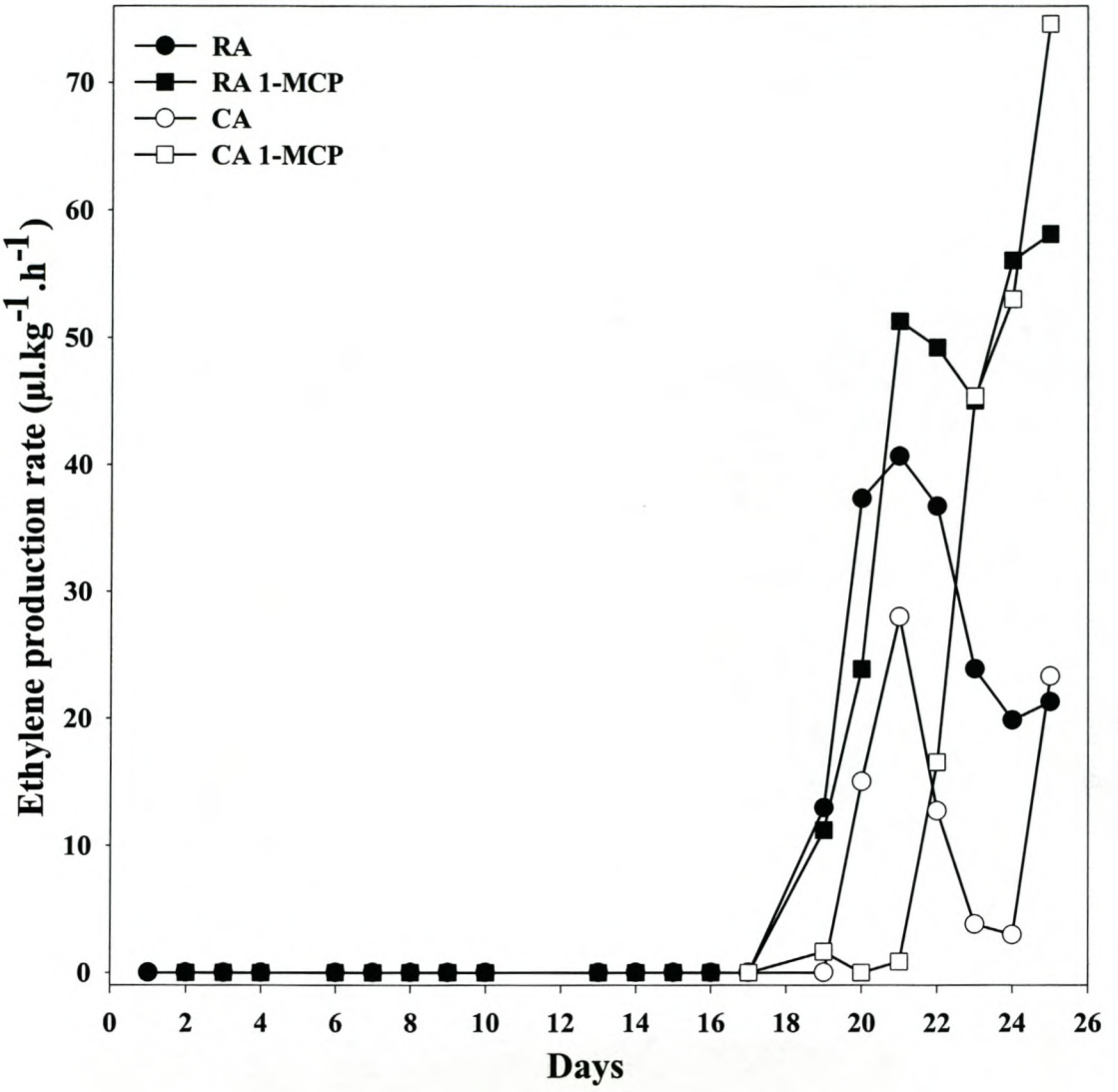

Fig. 6. Ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA), followed by 20°C in air until eating ripe. LSD values were calculated for the shelf life period (*LSD = 40.813*). *1-MCP treatment involved a 24 hour exposure at 500 nl.l^{-1} at room temperature immediately prior to cold storage.*

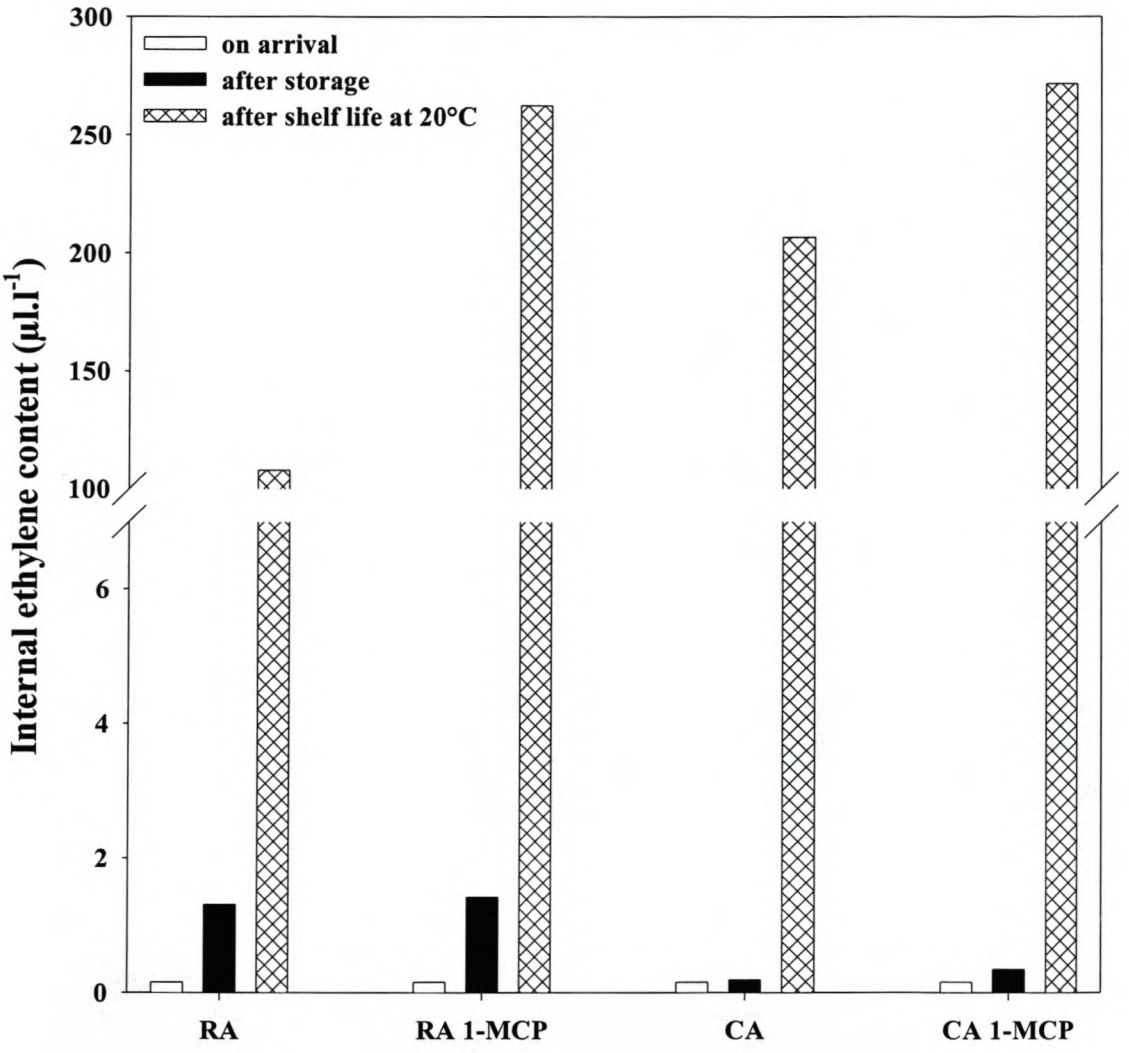

Fig. 7. Internal ethylene content ($\mu l.l^{-1}$) of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA), followed by 20°C in air until eating ripe. Measurements were taken initially on arrival, after 18 days storage (*LSD = 2.1647*) and after seven days of shelf life at 20°C (*LSD = 87.073*). *1-MCP treatment involved a 24 hour exposure at 500 nl.l^{-1} at room temperature immediately prior to cold storage.*

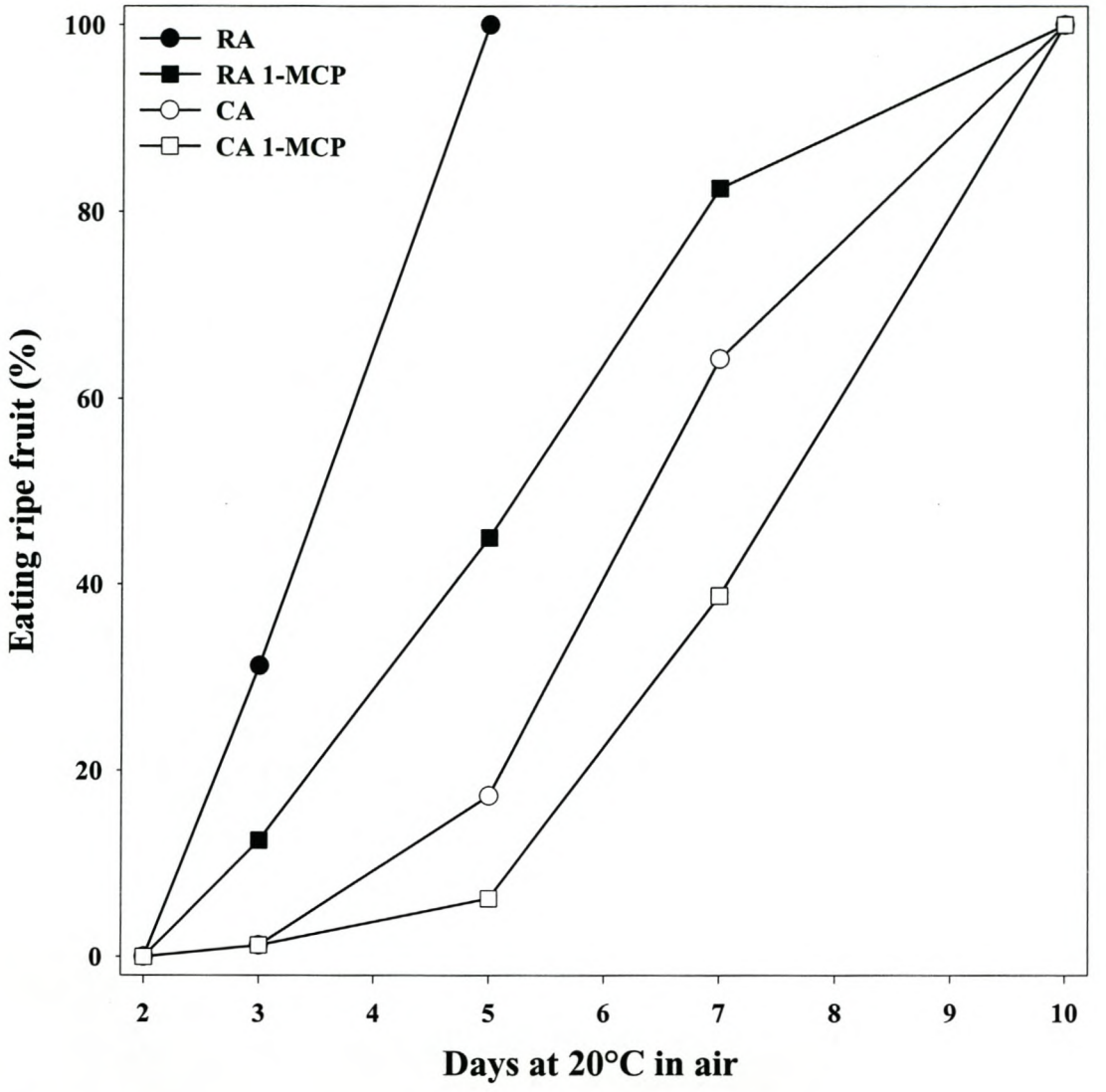

Fig. 8. Ripening rates of 'Hass' avocado fruit stored at 5.5°C for 18 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA), followed by 20°C in air until eating ripe and the percentages recorded. *1-MCP treatment involved a 24 hour exposure at 500 nl.l^{-1} at room temperature immediately prior to cold storage.*

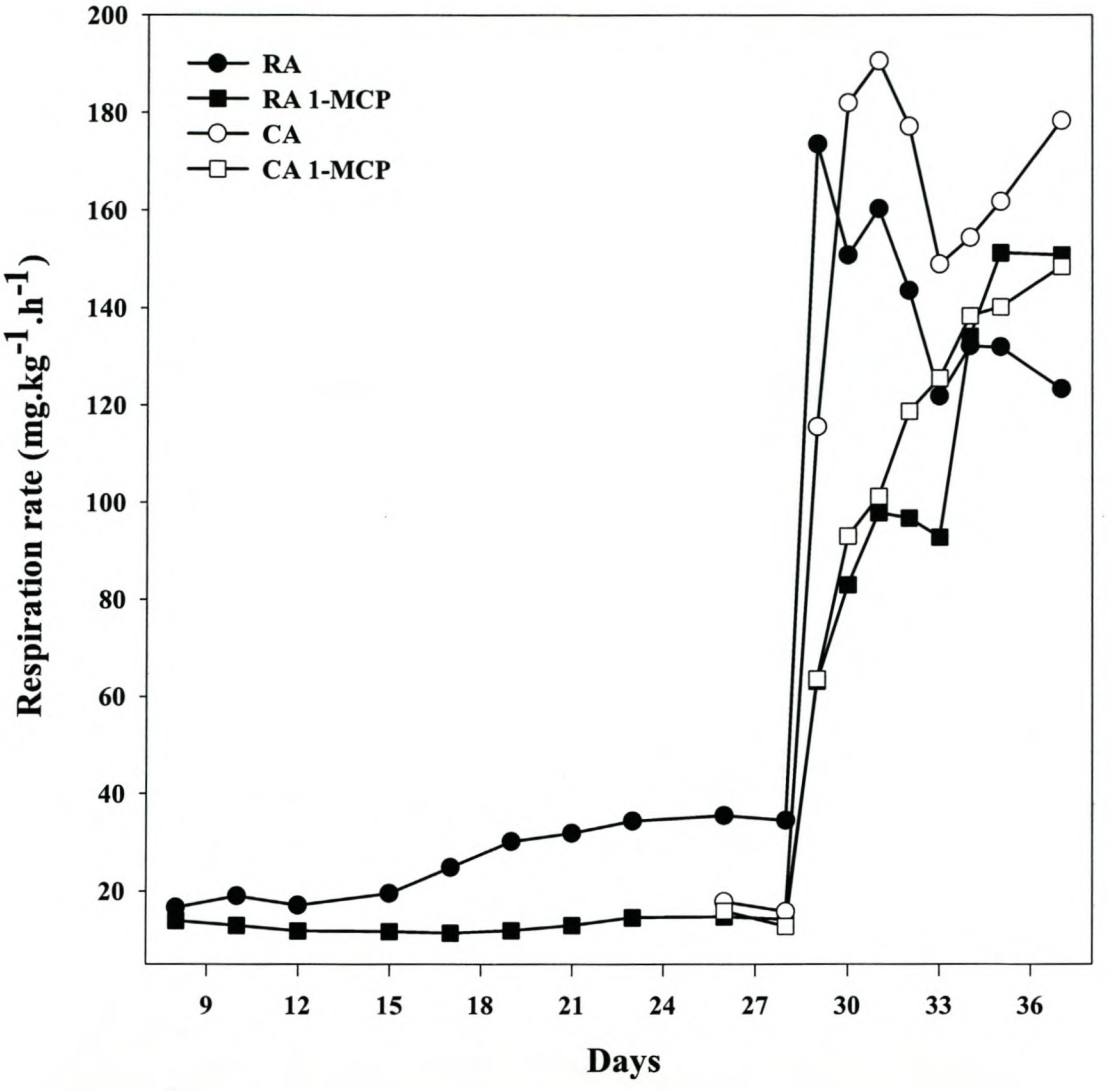

Fig. 9. Respiration rate (CO_2 evolved in $mg.kg^{-1}.h^{-1}$) of 'Fuerte' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days, followed by 20°C in air until eating ripe. LSD values were calculated after 25 days storage (*LSD = 2.6444*) and after CA finished including the shelf life period (*LSD = 40.396*). *1-MCP treatment involved a 16 hour exposure at 300 nl.l^{-1} at storage temperature five days after harvest.*

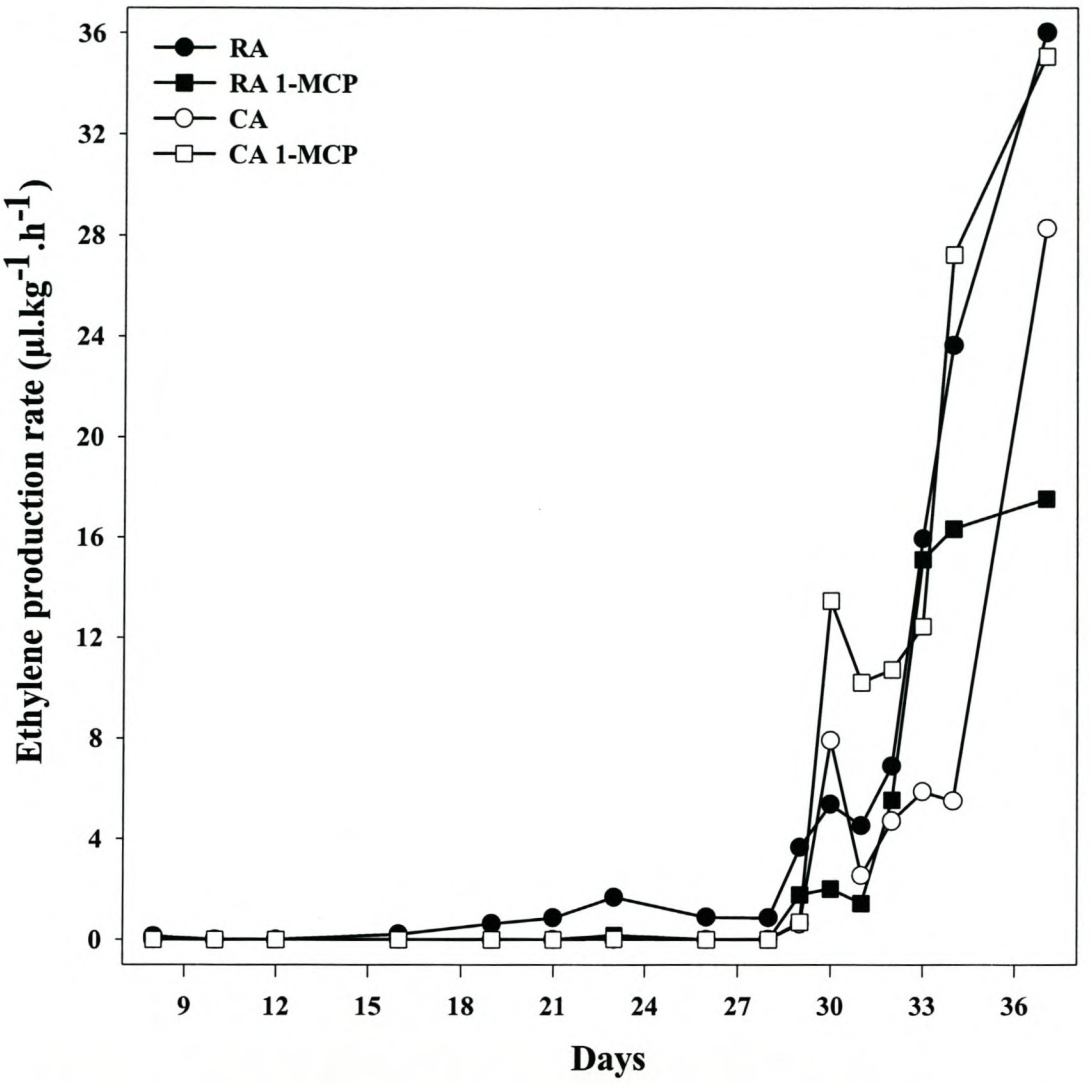

Fig. 10. Ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of 'Fuerte' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days, followed by 20°C in air until eating ripe (*LSD = 11.8147*). *1-MCP treatment involved a 16 hour exposure at 300 nl.l^{-1} at storage temperature five days after harvest.*

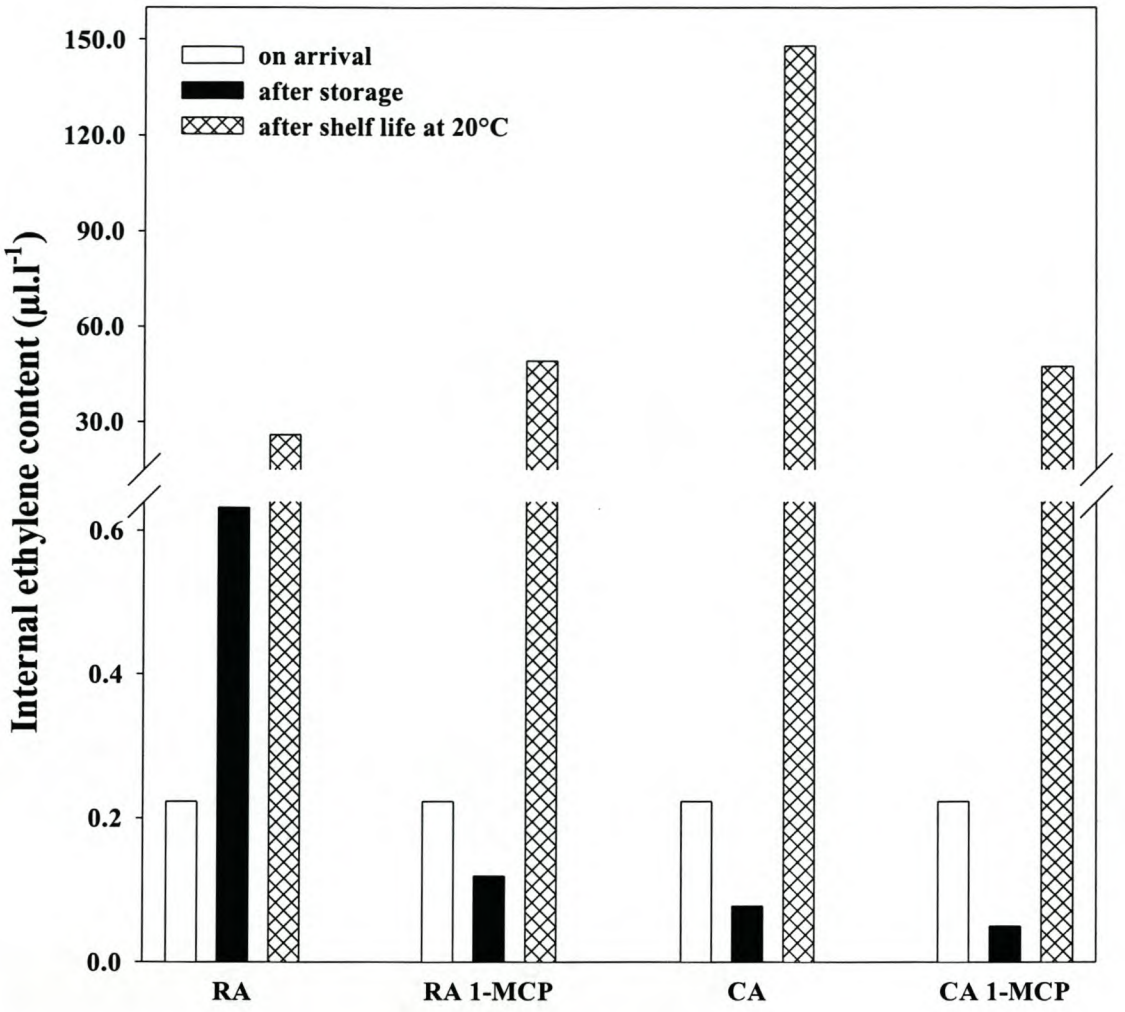

Fig. 11. Internal ethylene content ($\mu l.l^{-1}$) of 'Fuerte' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days, followed by 20°C in air until eating ripe. Measurements were taken on arrival, after 28 days storage (*LSD = 0.2569*) and after nine days shelf life at 20°C (*LSD = 82.855*). *1-MCP treatment involved a 16 hour exposure at 300 nl.l^{-1} at storage temperature five days after harvest.*

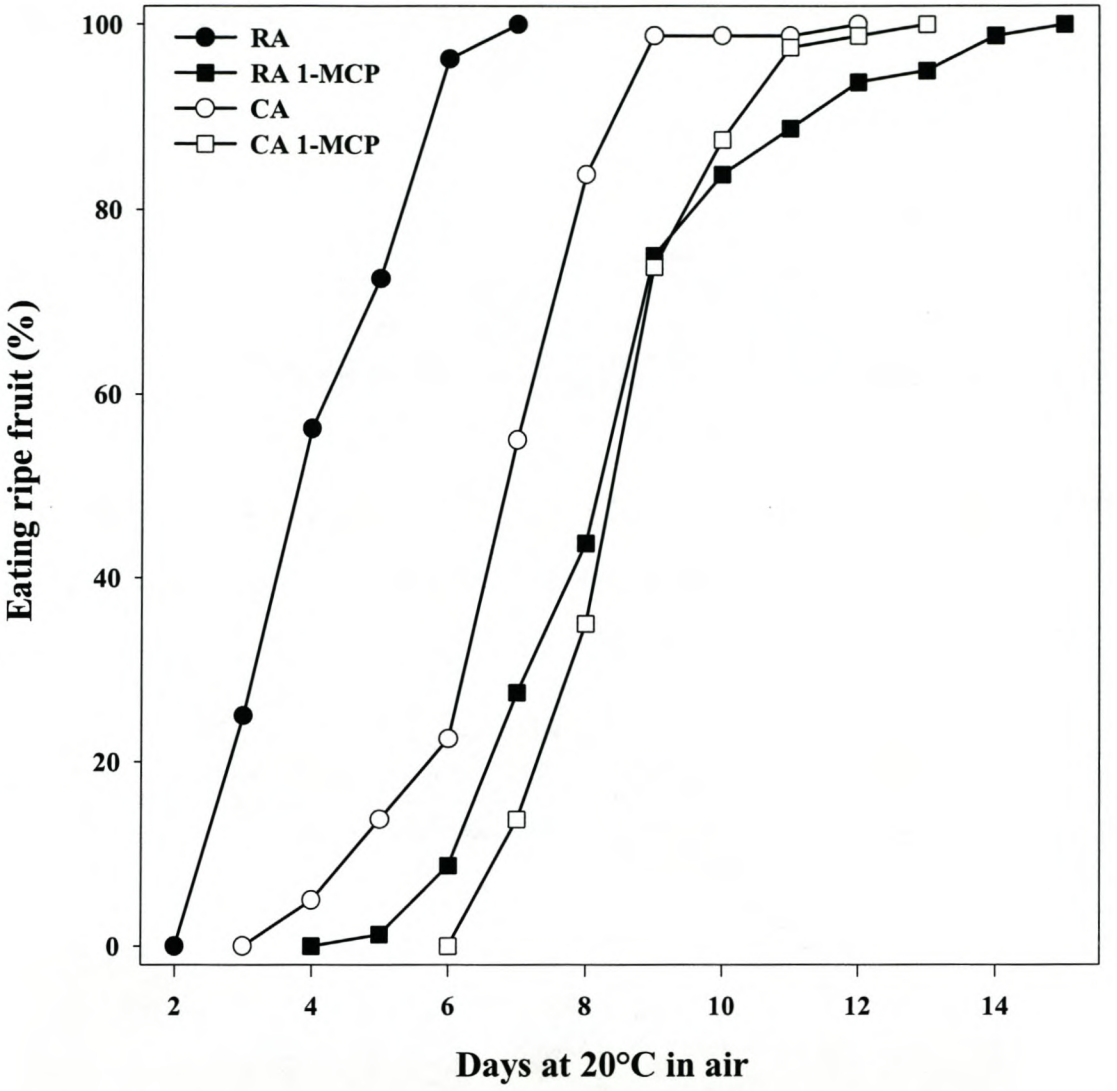

Fig. 12. Ripening rates of 'Fuerte' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days, followed by 20°C in air until eating ripe and the percentages recorded. *1-MCP treatment involved a 16 hour exposure at 300 nl.l^{-1} at storage temperature five days after harvest.*

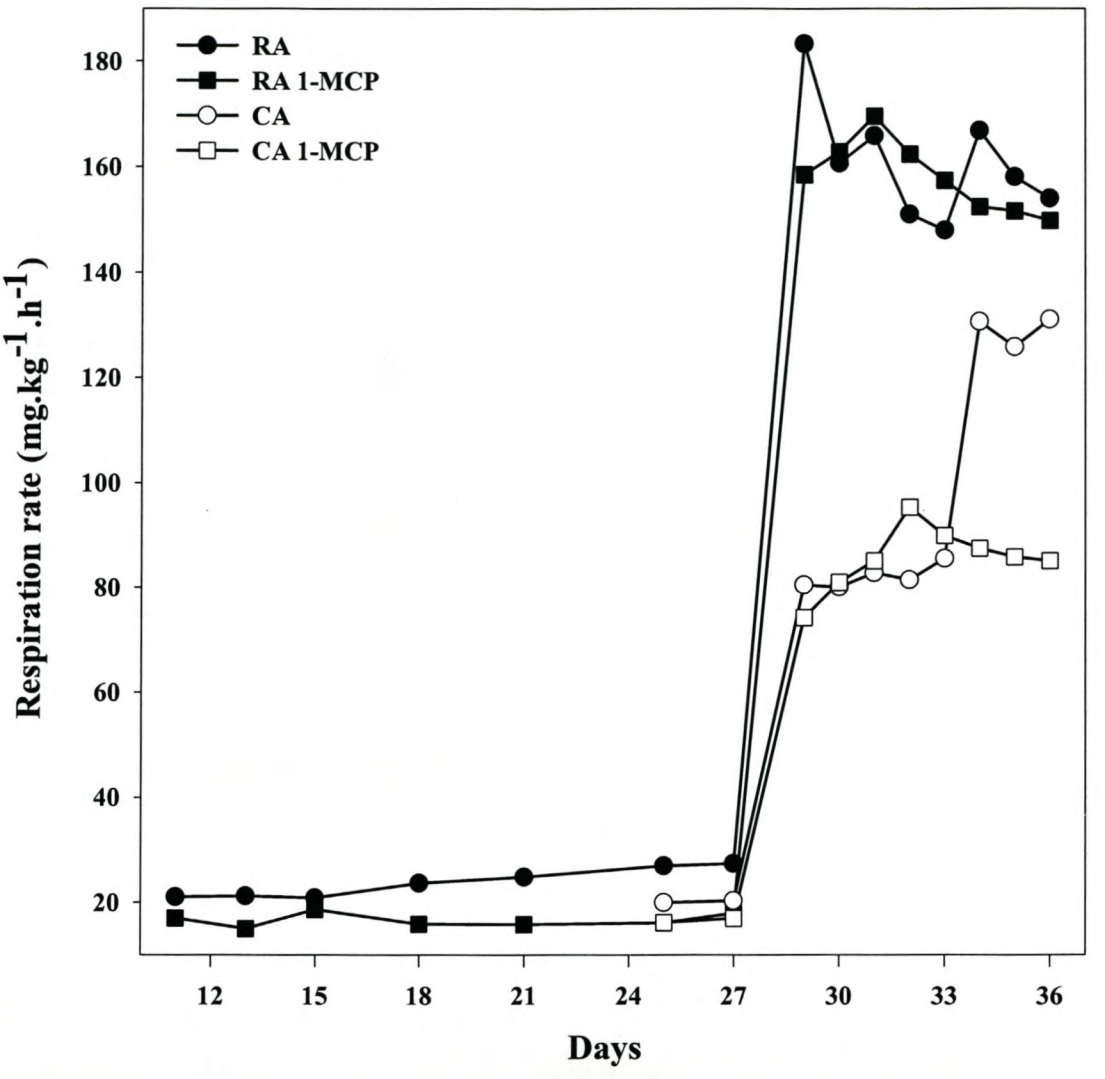

Fig. 13. Respiration rate (CO_2 evolved in $mg.kg^{-1}.h^{-1}$) of 'Hass' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days, followed by 20°C in air until eating ripe. LSD values were calculated after 25 days storage (*LSD = 6.0372*) and after CA finished including the shelf life period (*LSD = 50.316*). *1-MCP treatment involved a 16 hour exposure at 300 nl.l^{-1} at storage temperature five days after harvest.*

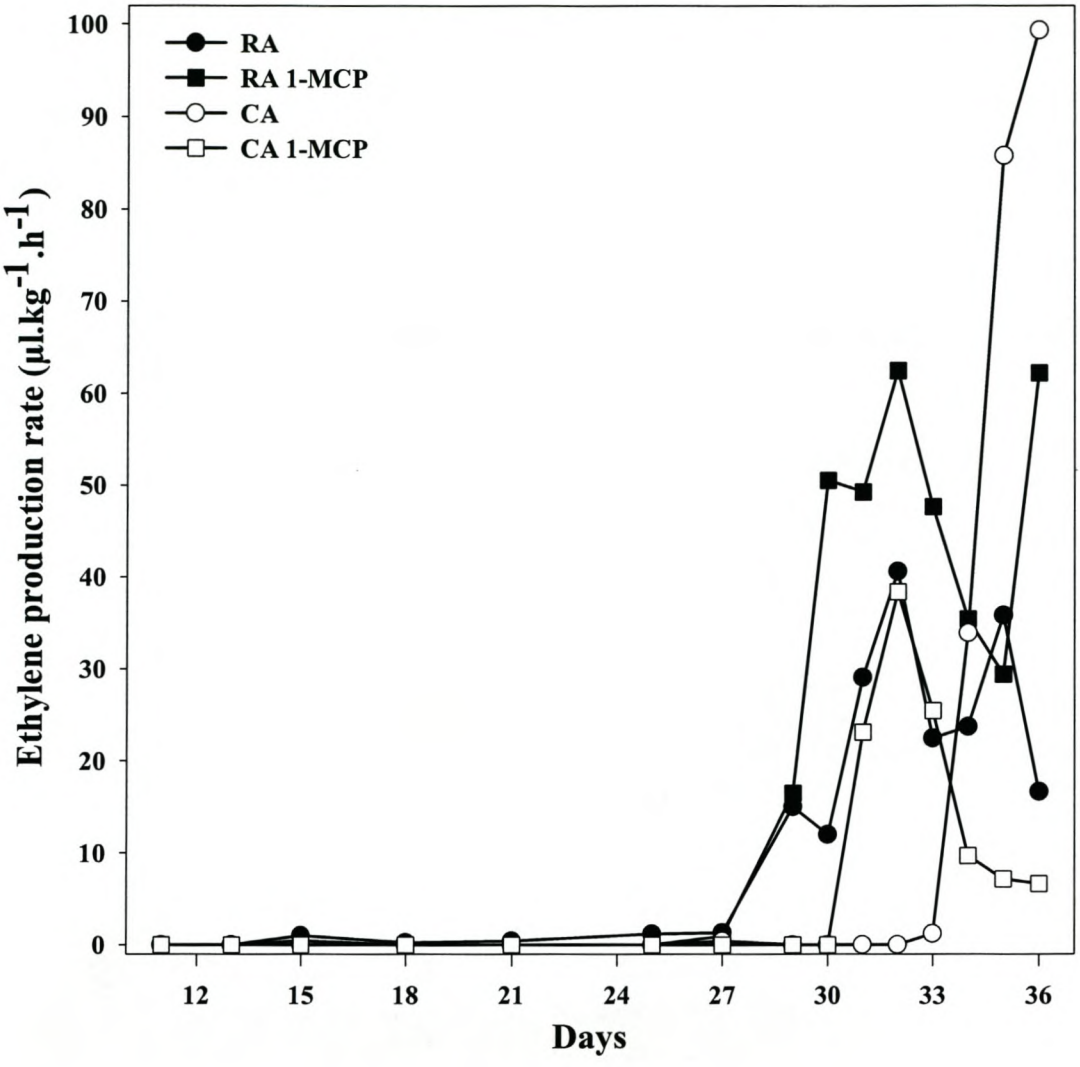

Fig. 14. Ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of 'Hass' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days, followed by 20°C in air until eating ripe (*LSD = 45.125*). *1-MCP treatment involved a 16 hour exposure at 300 nl.l^{-1} at storage temperature five days after harvest.*

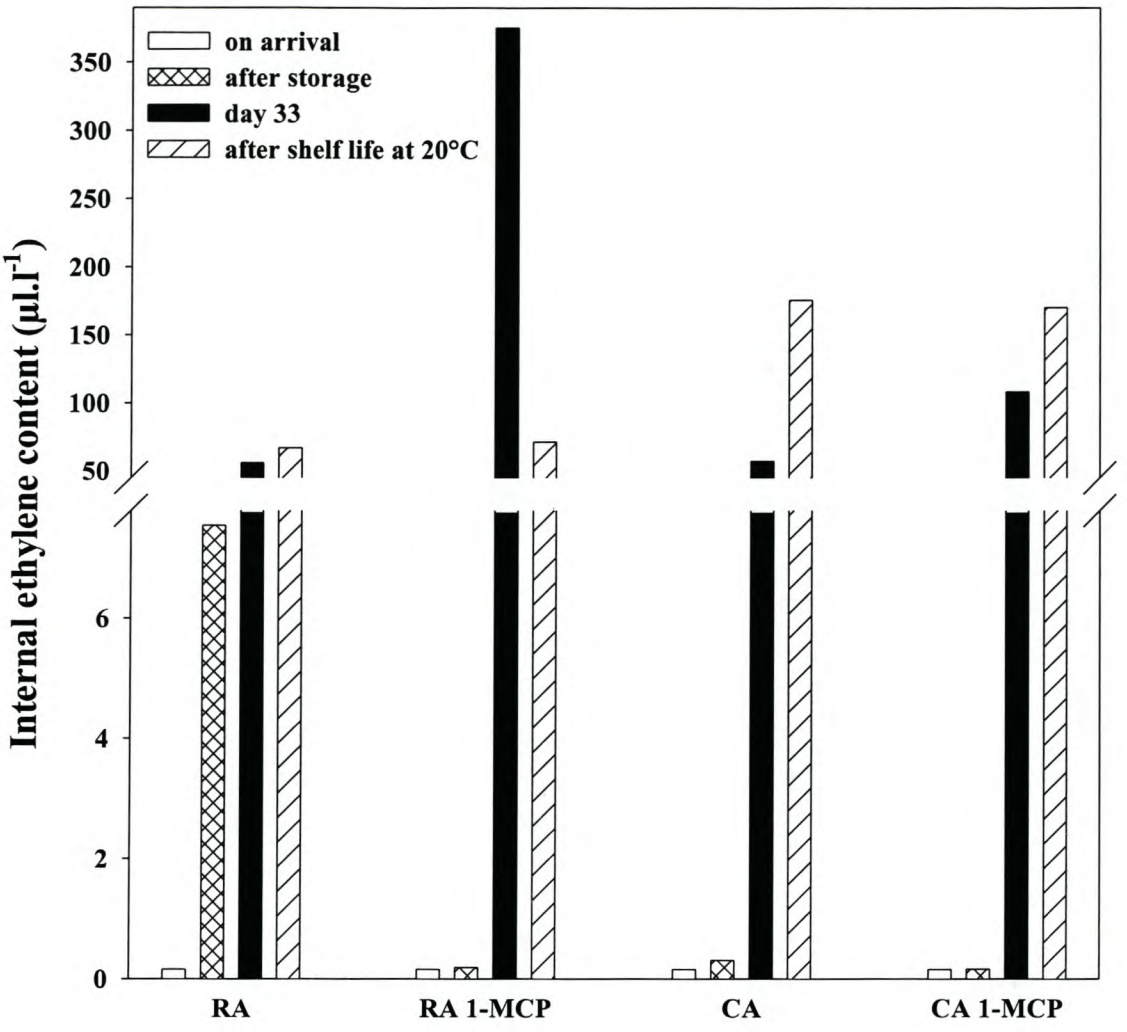

Fig. 15. Internal ethylene content ($\mu l.l^{-1}$) of 'Hass' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days, followed by 20°C in air until eating ripe. Measurements were taken on arrival, after 28 days storage (*LSD = 6.9519*), after five days shelf life at 20°C (*LSD = 98.794*) and after nine days shelf life at 20°C (*LSD = 54.074*). *1-MCP treatment involved a 16 hour exposure at 300 nl.l^{-1} at storage temperature five days after harvest.*

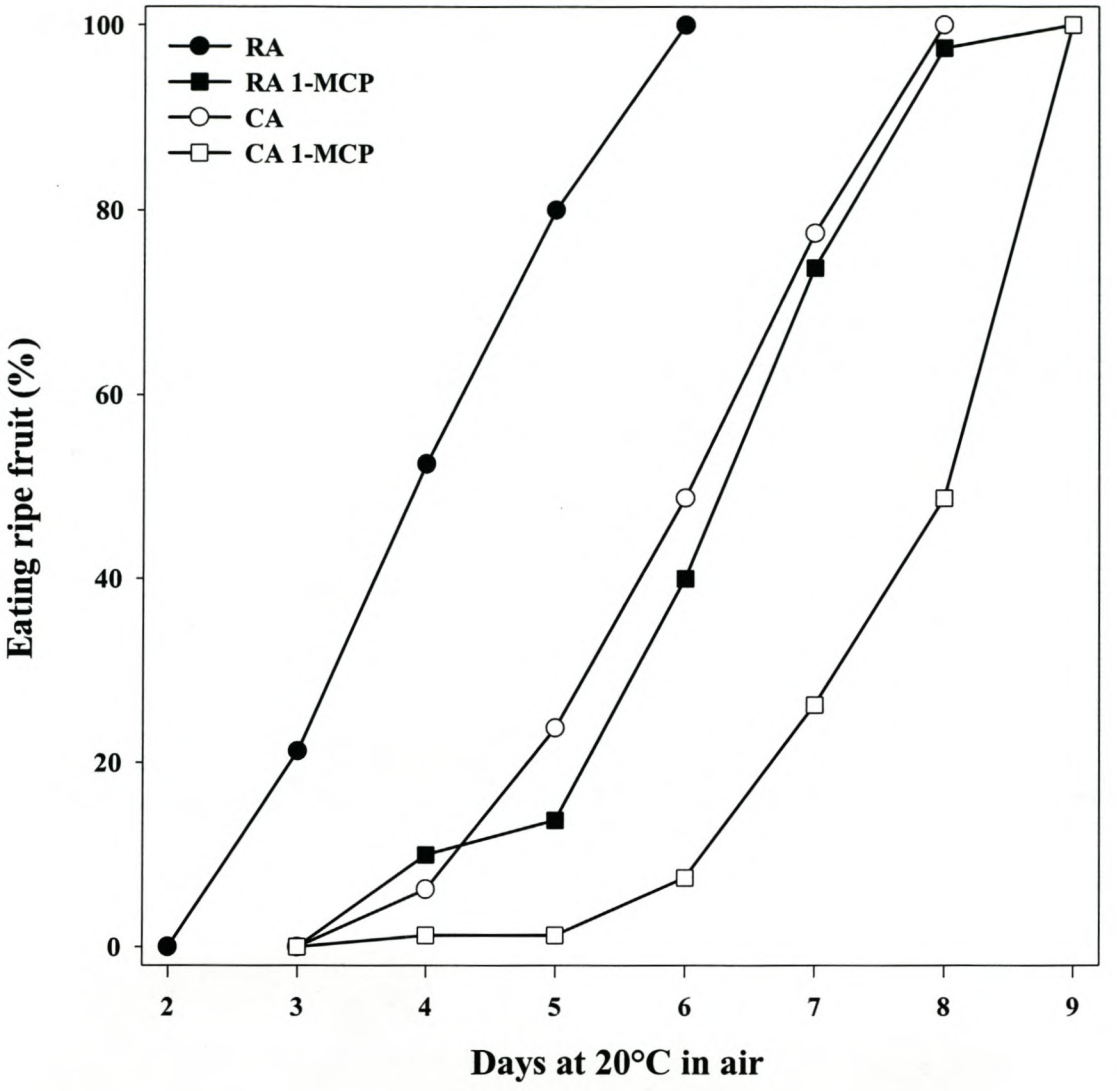

Fig. 16. Ripening rates of 'Hass' avocado fruit stored at 7°C for 28 days under regular atmosphere with 1-MCP (RA 1-MCP) or without 1-MCP (RA), or controlled atmosphere, with 1-MCP (CA 1-MCP) or without 1-MCP (CA). Storage under CA occurred for 18 of the 28 days after which fruit were stored in air for the balance of the 28 days, followed by 20°C in air until eating ripe and the percentages recorded. *1-MCP treatment involved a 16 hour exposure at 300 nl.l^{-1} at storage temperature five days after harvest.*

6. ARTICLE 4: Storage of 'Fuerte' and 'Hass' Avocados at Two Temperatures and Two Relative Humidities

STORAGE OF 'FUERTE' AND 'HASS' AVOCADOS AT TWO TEMPERATURES AND TWO RELATIVE HUMIDITIES

Abstract

Throughout the industry the handling technologies for avocados are being seriously considered so as to meet the demands of the export market for high quality fruit. 'Fuerte' and 'Hass' avocados were stored at the commercial temperature for the stage of the season and a chilling temperature, both in combination with a high and a low relative humidity level for 28 days. Thereafter the fruit were stored in air until the eating ripe stage was reached. Once eating ripe no more than 44% of the fruit within a treatment for either of the cultivars were sound. The fruit were mostly affected by the internal disorders, grey pulp and vascular browning, while the 'Hass' avocados also had prominent decay damage. Although previous research has shown that high RH can restrict chilling injury and extend shelf life of avocados this was not apparent in our work. However, more work over a wider range of cultivars, relative humidity levels and temperature levels may prove to be more successful.

Introduction

Avocados from South Africa due for the export market are more often than not in transit for no fewer than 30 days from the time of harvest (Couey, 1982). Due to these adverse conditions to which the fruit are exposed by being in cold storage for extended periods it is not unusual for the fruit to arrive at the market at the incorrect stage of maturity. With these extended storage periods chilling injury also poses a very real problem for avocado storage (Couey, 1982) and it is for this reason that very low temperature storage is not acceptable. However, higher relative humidity (RH) levels (95%) during storage have been proven to restrict chilling injury (Chien et al., 1998) and ripening of different fruit (Adato and Gazit, 1974; Pesis et al., 2000; Xue et al., 1996).

Relative humidity is an environmental factor which influences the rate of water loss of whole plants or excised plant organs (Forney and Brandl, 1992). Physiological processes such as cell expansion, growth, photosynthesis and senescence are affected

by this water loss (Forney and Brandl, 1992). The difficulty in controlling and measuring RH has meant that it is an inconvenience and its role in the physiology of plants has generally been ignored (Gaffney, 1978). Salt solutions have previously been shown to be very effective in controlling humidity but according to Solomon (as reported by Forney and Brandl, 1992) the problem is that a different salt solution is needed for different humidity levels. This, coupled with the fact that RH varies with temperature changes (Forney and Brandl, 1992), poses more of a problem than an inconvenience. Equilibrium RH can also be formed with nonsaturated solutions such as glycerol (Forney and Brandl, 1992). Glycerol solutions are easy to mix, the exact composition for a specific RH can be determined and it is relatively inexpensive (Forney and Brandl, 1992).

It must be remembered that increased RH may have a positive influence on delaying fruit and vegetable ripening but if the moisture content of the air reaches saturation level (100% RH) it could provide an ideal environment for decay organisms to develop. For this reason Hardenburg et al. (1986) recommend for storage of fresh produce RH between 85% and 95%, which presents a balance between microbial spoilage and weight loss (Shirazi and Cameron, 1992). We hypothesise that storage of 'Fuerte' and 'Hass' avocados at high RH levels will allow fruit to be stored at lower than normal temperatures without causing chilling injury symptoms, and will delay fruit ripening.

Materials and Methods

Experimental set up: 'Fuerte' and 'Hass' avocado fruit were harvested on the 12th of June 2002 and transported at 5.5°C and 7°C, respectively, to the University of Stellenbosch by Westfalia exporters. Fruit size was count 14 (266 - 305 g) and was intended for the export market.

The fruit were sorted on the 21st June 2002 and all damaged fruit were discarded. The fruit were stored at the recommended temperature for that stage of the season and at a chilling temperature in combination with two different RH levels for four weeks. The different treatments were: low temperature and high RH (LT-HRH) ('Fuerte' at 3°C, 'Hass' at 5.5°C and 100% RH), high temperature and high RH (HT-HRH) ('Fuerte' at

5.5°C, 'Hass' at 7°C and 100% RH), low temperature and low RH (LT-LRH) ('Fuerte' at 3°C, 'Hass' at 5.5°C and 75% RH) and high temperature and low RH (HT-LRH) ('Fuerte' at 5.5°C, 'Hass' at 7°C and 75% RH). Thereafter, temperatures were increased to 20°C until all fruit were eating ripe to simulate a shelf life period in air.

Fruit were placed in 5 L buckets and connected to humidified air supplied via flow boards. Flow rates were about 450 $ml.min^{-1}$ during storage. The experimental design was a randomised block with four treatments each consisting of six replications with five fruit each.

A representative set of 20 fruit was taken initially and evaluated for firmness prior to the fruit being treated. During the shelf life period the fruit were removed for disorder evaluation as they reached the eating ripe stage. This was assessed by gently squeezing the fruit by hand.

Maturity indices

Firmness. Readings were taken on opposite sides of the peeled fruit with a penetrometer (Southtrade fruit pressure tester, FT 327, Alphonsine, Italy) fitted with a 5 mm tip.

Moisture content. Moisture content was measured only initially when the fruit arrived. It was only done once as moisture content does not change much during the storage period and is used as a maturity index for harvest. Moisture content was determined by the method described by Swarts (1978). The fruit was cut in half and the pip removed. The fruit was grated at the cut surface and weighed. The sample was placed in a microwave on high for two minutes after which it was reweighed. The sample was replaced in the microwave for a further two minutes and reweighed, and the process repeated until a constant mass was achieved. The difference between the initial mass of the sample and the final mass of the sample as a percentage of the initial mass of the sample gave the moisture content of the fruit. This was done on three fruit. For each new two minute cycle a beaker of cold water was placed in the microwave with the fruit sample, to prevent burning of the sample.

Disorders. Fruit were evaluated when eating ripe during the shelf life period. Fruit were rated for external disorders: chilling injury, black cold, Dothiorella / Colletotrichum complex (D/C) and lenticel damage. The fruit were then cut in half and allowed to stand for 10 minutes so any internal disorders could become visible. The fruit were rated for internal disorders: pulp spot, grey pulp and vascular browning. The decay which was rated was: stem-end rot, internal anthracnose and external anthracnose. The statistics for the disorders was calculated as a percentage of the total number of fruit per replication evaluated for disorders.

Ripening rates. As the fruit were removed from the shelf life period the number of fruit per treatment and days at 20°C until ripe were recorded.

Statistical Analysis. Analysis of variance (ANOVA) of the main effects and LSD values with a significance level of 5% were obtained using Statistical Analysis Systems (SAS). Presented data points are the means of the four replications ±SE.

Results

Expt 1: 'Fuerte'

At the start of the experiment the fruit had a mean moisture content of 67.0% and were therefore stored at 5.5°C (Hardy et al., undated). On arrival the fruit had a mean firmness of 10.6 kg.

Disorders. There was no significant difference between treatments in the percentage of sound fruit and values ranged between 16.7 - 43.3% with the fruit stored under LT-LRH having the highest percentage (Table 1). The external disorders which occurred were chilling injury and lenticel damage. There were no significant differences between the treatments for lenticel damage (Table 1). The fruit stored under LT had significantly higher chilling injury (21.7%) than the fruit stored under HT (2.8%) (Table 2).

There were no significant differences between treatments in the percentage pulp spot and its occurrence was no higher than 4.3% within a treatment (Table 1). The fruit stored under HT-LRH had the significantly highest occurrence of vascular browning

(60.0%) and there were no significant differences between the remaining treatments (Table 1). The fruit stored under LT had significantly higher grey pulp (50.1%) than the fruit stored under HT (25.0%) (Table 2). Similarly the fruit stored under HRH had significantly higher grey pulp (50.0%) than the fruit stored under LHR (25.0%) (Table 3). Stem-end rot and external anthracnose were less prominent and the highest occurrence was 13.3% external anthracnose (Table 1) but there was a significantly higher occurrence of internal anthracnose in the fruit stored under LT (6.7%) than the fruit stored under HT (0.3%) (Table 2).

Ripening rates. The fruit stored at 3°C (LT) generally had a slower ripening pattern than the fruit stored at 5.5°C (HT) (Fig. 1). At both temperatures the fruit stored under 75% RH ripened slower than those fruit stored under 100% RH.

Expt 2: 'Hass'

At the start of the experiment the fruit had a mean moisture content of 77.0% and were therefore stored at 7°C (Hardy et al., undated). On arrival the fruit had a mean firmness of 12.1 kg.

Disorders. The percentage sound fruit was no higher than 26.7% for any of the treatments and there was no significant difference between treatments (Table 4). Chilling injury was the only external disorder which occurred, and at levels lower than 5% with no significant differences between treatments (Table 4).

The occurrence of grey pulp was significantly higher in the fruit stored under LT (33.3%) than the fruit stored under HT (13.3%) (Table 2). There was no significant difference in the occurrence of vascular browning but levels as high as 50% did occur (Table 4).

The fruit stored under HT-LRH had the highest level of external anthracnose (30.0%) but only significantly higher than the fruit stored under LT-LRH (Table 4). The fruit stored under LT had the significantly highest levels of stem-end rot (21.7%) and internal anthracnose (18.3%) (Table 2). Similarly the fruit stored under HRH had the significantly highest levels of stem-end rot (22.2%) (Table 3).

Ripening rates. The fruit stored at 5.5°C (LT) had a distinctly slower ripening pattern than the fruit stored at 7°C (HT) (Fig. 2). Of the fruit stored at 5.5°C, those stored under 100% RH (HRH) ripened slower than those fruit stored under 75% RH (LRH).

Discussion and Conclusion

Disorders. None of the parameters significantly affected the percentage sound 'Fuerte' fruit (Table 1). Temperature did, however, significantly affect the level of chilling injury and thus the fruit stored at the lower temperature were more damaged (Table 2). 'Fuerte' avocados, which are known to be susceptible to pulp spot, were hardly affected by the disorder. In contrast to that, grey pulp was far more prominent and was significantly influenced by both higher RH levels and lower temperatures (Tables 2 and 3). There was a significant interaction between temperature and RH for the occurrence of vascular browning, thus the main effects cannot be discussed (Table 1).

'Hass' avocados are known to be very resistant against disorders even after extended cold storage in air. In our experiment, however, the fruit resisted disorders very poorly as can be seen by the fact that none of the treatments had more than 27% sound fruit. Neither temperature nor RH had a significant influence on the percentage of sound fruit (Table 4). Grey pulp was significantly more prominent at the lower storage temperature (Table 2) while there was no significant temperature or RH effect on the level of vascular browning (Table 4). Stem-end rot and internal anthracnose were significantly more prominent at the lower temperature (Table 2). In addition, stem-end rot was also significantly more prominent at the higher RH level (Table 3).

Chilling injury of avocados commonly results in pitting and blackening of the exocarp as well as mesocarp discolouration (Couey, 1982). The latter disorder can be classified into two forms: pulp spot, which is the discoloration of the vascular bundles (most evident when severed) resulting in a grey spot on the flesh, and grey pulp, which is a grey discolouration of the mesocarp (Swarts, 1984). These browning reactions are due to the activity of polyphenoloxidase (PPO) and its role in the oxidation of o-diphenols to o-quinones. This commonly occurs when cells rupture

and the phenolic acids are oxidised to quinones which produce a brown colour once polymerised (Torres et al., 1987). Donkin (1995) found that 'Fuerte' and 'Hass' avocados are most susceptible to chilling injury at the climacteric peak and for this reason White et al. (2001) suggested maintenance of low ethylene levels in the storage environment for avocados.

Peel pitting and other blemishes of chilling sensitive cucumbers were restricted by reducing water loss using high RH levels (Chien et al., 1998) but care must be taken because RH levels nearing 100% could result in decay. This has been found on 'Red Bell' peppers (Polderdijk et al., 1993). RH levels as high as 90% were able to improve quality of litchis by restricting browning and anthocyanin loss (Jiang and Fu, 1999).

Storage of fruit and vegetables under MAP conditions can very often result in the air becoming water saturated and providing the ideal environment for development of decay organisms. Rodov et al. (1995) found that the RH within a package could be decreased with sodium chloride, a hygroscopic material, to between 88 to 97% depending on the amount used and the weight of the fruit in the package. This treatment was able to reduce the level of decay during 'Red Bell' pepper storage and thus extend the shelf life.

Ripening rates. For both the 'Fuerte' and 'Hass' experiments temperature had the primary influence as the lower temperature delayed fruit ripening (Fig. 1 and 2). However, in 'Fuerte' the ripening was delayed by storage at 75% RH whereas the ripening of 'Hass' was delayed by storage at 100% RH.

Previous research has shown that at lowered RH the ripening of mangos (Pesis et al., 2000), 'Fuerte' avocados (Adato and Gazit, 1974), 'Hass' avocados (Adato and Gazit, 1974) and 'Giant Cavendish' bananas (Xue et al., 1996) was accelerated. This can be ascribed to increased respiration and ethylene production rates at the lower RH level (Pesis et al., 2000).

Thus, to conclude, the storage of 'Fuerte' and 'Hass' avocados at chilling-inducing temperatures in combination with high RH did not show much promise. Both

cultivars were affected primarily by internal disorders and decay disorders. The 'Hass' fruit shelf life was extended by the storage at lower temperature and higher RH but this was outweighed by the poor quality of the fruit.

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Table 1

Internal and external disorders and the significance levels of temperature and relative humidity of 'Fuerte' avocado fruit stored at 5.5°C for nine days after harvest then stored at the commercial storage temperature of 5.5°C (HT) or a chilling temperature of 3°C (LT) in combination with either 100% (HRH) or 75% (LRH) relative humidity for 28 days. Fruit were ripened at 20°C in air until eating ripe.

	Sound fruit (%)	Lenticel damage (%)	Pulp spot (%)	Vascular browning (%)	Stem-end rot (%)	External anthracnose (%)
LT-HRH	40.0 ns[z]	3.3 ns	0.0 ns	6.7 b	0.0 ns	6.7 ns
LT-LRH	43.3	3.3	0.0	13.3 b	3.3	0.0
HT-HRH	33.3	0.0	4.3	20.0 b	10.5	0.0
HT-LRH	16.7	0.0	0.3	60.0 a	3.0	13.3
LSD	*29.988*	*6.9532*	*4.685*	*15.856*	*11.034*	*13.906*
Temperature	0.1167	0.1727	0.1573	0.0001	0.1893	0.4877
Relative Humidity	0.5194	1.0000	0.2224	0.0003	0.5837	0.4877
Temperature*Relative humidity	0.3370	1.0000	0.2224	0.0056	0.1631	0.0466

[z] Means separation within columns using least significant difference (0.05)

Table 2

Significance levels as influenced by temperature of 'Fuerte' and 'Hass' avocado fruit cold stored for nine days after harvest then stored at the commercial storage temperature ('Fuerte': .5°C; 'Hass': 7°C) (HT) or a chilling temperature ('Fuerte': 3°C; 'Hass': 5.5°C) (LT) in combination with either 100% (HRH) or 75% (LRH) relative humidity for 28 days. Fruit were ripened at 20°C in air until eating ripe.

	'Fuerte'			'Hass'		
	Chilling injury (%)	Grey pulp (%)	Internal anthracnose (%)	Grey pulp (%)	Stem-end rot (%)	Internal anthracnose (%)
LT	21.7 a[z]	50.1 a	6.7 a	33.3 a	21.7 a	18.3 a
HT	2.8 b	25.0 b	0.3 b	13.3 b	6.7 b	2.8 b
LSD	*15.986*	*20.092*	*6.2347*	*18.132*	*12.089*	*10.526*

[z] Means separation within columns using least significant difference (0.05)

Table 3

Significance levels as influenced by relative humidity of 'Fuerte' and 'Hass' avocado fruit cold stored for nine days after harvest then stored at the commercial storage temperature ('Fuerte': 5.5°C; 'Hass': 7°C) (HT) or a chilling temperature ('Fuerte': 3°C; 'Hass': 5.5°C) (LT) in combination with either 100% (HRH) or 75% (LRH) relative humidity for 28 days. Fruit were ripened at 20°C in air until eating ripe.

	'Fuerte'		'Hass'	
	Grey pulp (%)		Stem-end rot (%)	
HRH	50.0	a[z]	22.2	a
LRH	25.0	b	6.2	b
LSD	*20.092*		*12.089*	

[z] Means separation within columns using least significant difference (0.05)

Table 4

Internal and external disorders and the significance levels of temperature and relative humidity of 'Hass' avocado fruit stored at 7°C for nine days after harvest then stored at the commercial storage temperature 7°C (HT) or a chilling temperature 5.5°C (LT) in combination with either 100% (HRH) or 75% (LRH) relative humidity for 28 days. Fruit were ripened at 20°C in air until eating ripe.

	Sound fruit (%)	Chilling injury (%)	Pulp spot (%)	Vascular browning (%)	External anthracnose (%)
LT-HRH	26.7 ns[z]	3.3 ns	0.0 ns	43.3 ns	10.0 ab
LT-LRH	20.0	0.0	3.3	36.7	0.0 b
HT-HRH	20.0	4.7	0.0	50.0	16.7 ab
HT-LRH	20.0	3.2	0.3	46.7	30.0 a
LSD	*29.17*	*6.778*	*4.9265*	*27.726*	*27.375*
Temperature	0.7396	0.3391	0.3798	0.3858	0.0622
Relative Humidity	0.7396	0.3054	0.2853	0.6006	0.8593
Temperature*Relative Humidity	0.7396	0.6942	0.3798	0.8610	0.2232

[z] Means separation within columns using least significant difference (0.05)

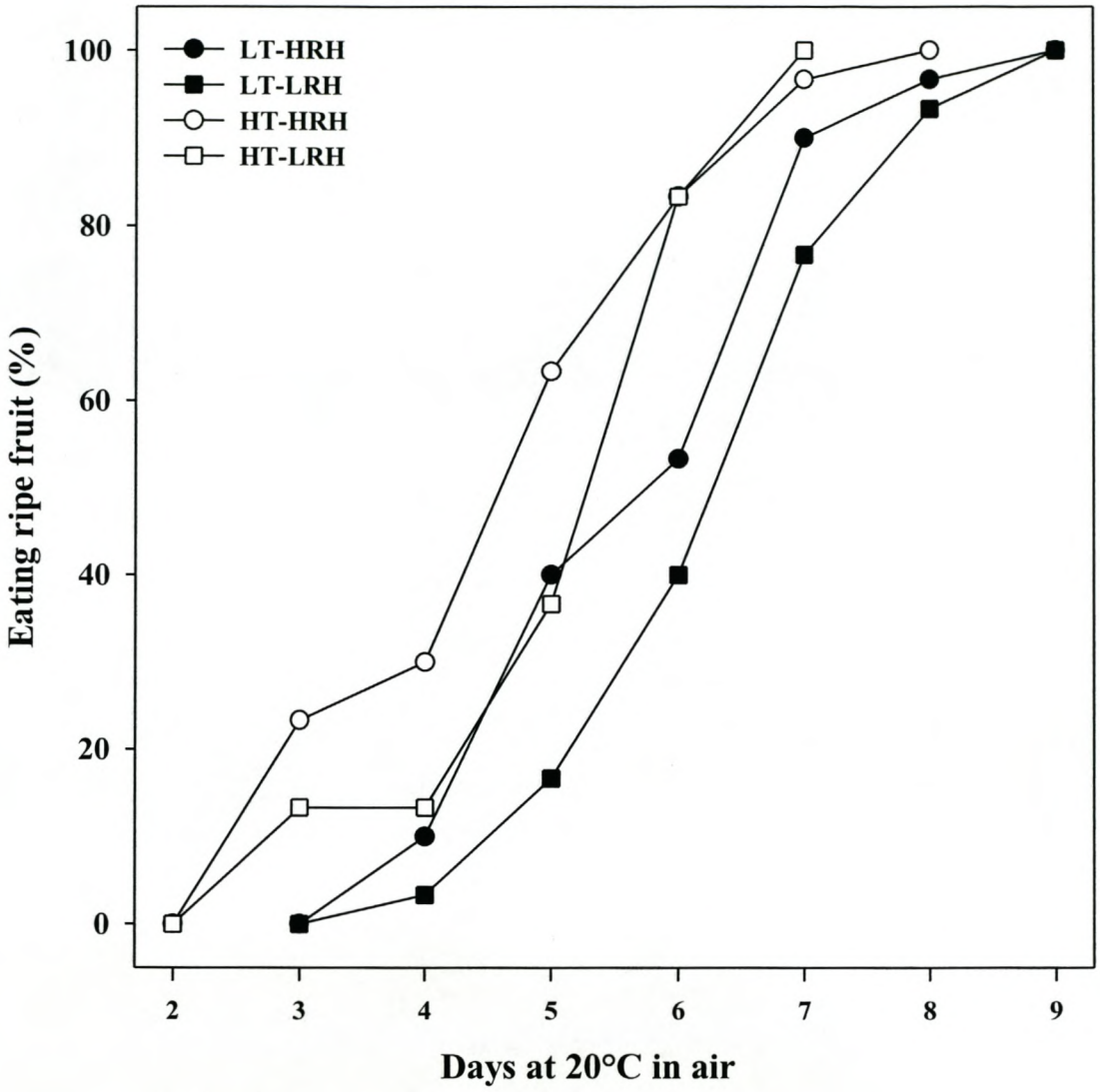

Fig. 1. Ripening rates of 'Fuerte' avocado fruit stored at 5.5°C for nine days after harvest then stored at the commercial storage temperature of 5.5°C (HT) or a chilling temperature of 3°C (LT) in combination with either 100% (HRH) or 75% (LRH) relative humidity for 28 days. Fruit were ripened at 20°C in air until eating ripe.

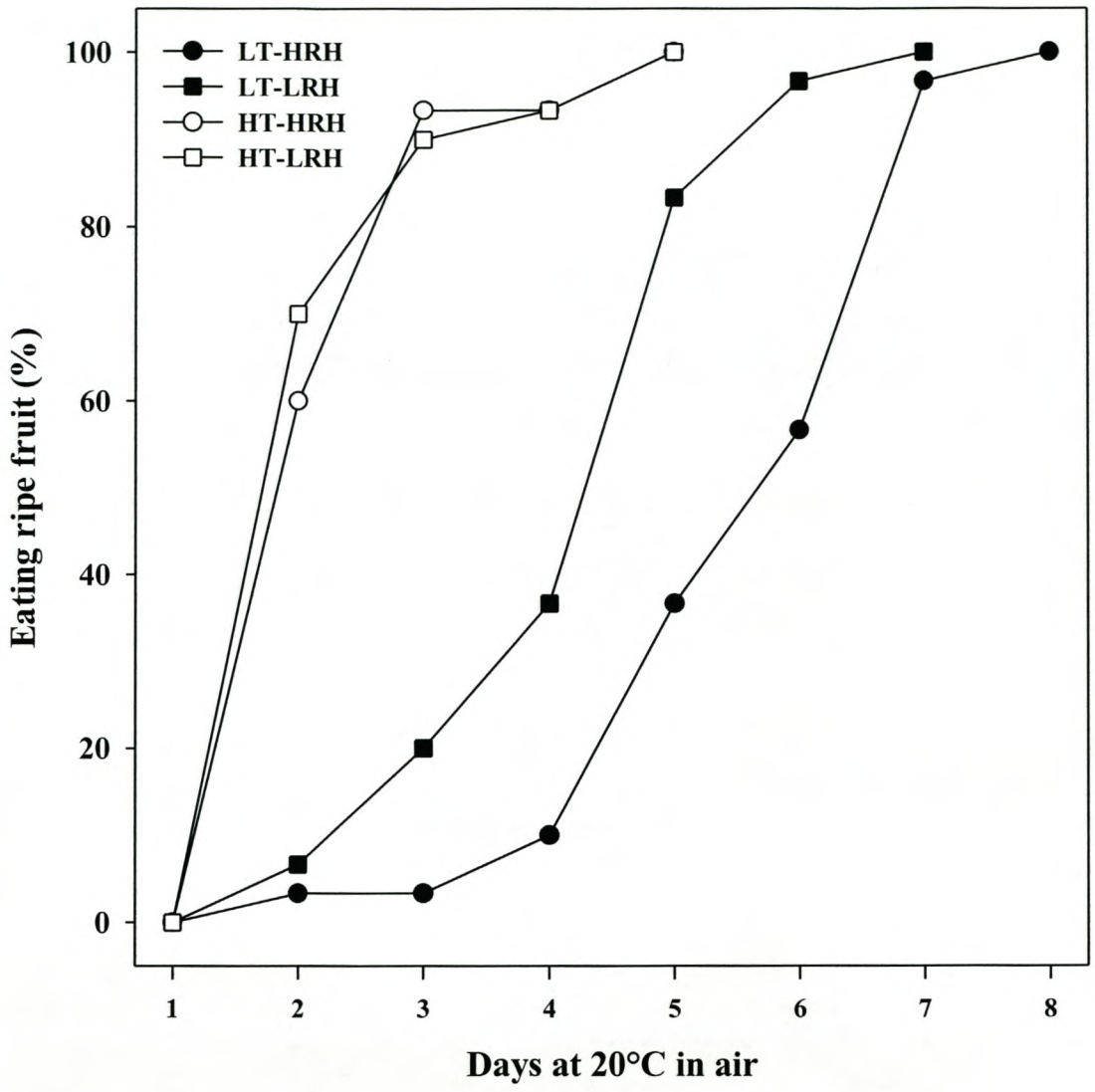

Fig. 2. Ripening rates of 'Hass' avocado fruit stored at 7°C for nine days after harvest then stored at the commercial storage temperature 7°C (HT) or a chilling temperature 5.5°C (LT) in combination with either 100% (HRH) or 75% (LRH) relative humidity for 28 days. Fruit were ripened at 20°C in air until eating ripe.

7. CONCLUSION

During season one the 'Songold' and 'Angeleno' plums stored under the single high temperature regime under CA performed as well or better than the commercially used dual temperature storage in air. This was evident by the monitoring of firmness, colour development, total soluble solids, titratable acidity and disorder development after storage and after a shelf life period in air.

The adjustment of the storage regimes, during season two, to mirror the movement of the fruit from harvest to the consumer meant that despite the single temperature storage during the shipping time the two temperature regimes used were very similar. For this reason it was found that the fruit stored under CA performed better than the fruit stored in air, regardless of temperature regime. Most noticeable was the fact the there was less GB in the 'Songold' stored under CA.

Kruger et al. (2001) found 'Songold' and 'Angeleno' plums to be suppressed climacteric in nature and our data confirmed this. Thus due to the slow ripening rate of these plum cultivars the necessity for CA storage could be completely negated by a strictly controlled dual temperature storage regime. Furthermore, the use of CA storage at a higher temperature regime than is commercially used may be more beneficial for the export storage of climacteric plum cultivars which display stronger ripening rates.

The avocado industry has gained far better use from CA storage than the plum industry especially with regard to retaining fruit firmness and quality while still extending the shelf life of the fruit. This has been most beneficial for a cultivar such as 'Fuerte' which, under extended cold storage, delivers very poor quality. With the long distances between the harbour and most of the avocado producing areas in South Africa the fruit remain on the truck for up to three days before being loaded at the harbour. Many research experiments identified this time period as ideal to utilise as a CO_2 shocking period for the fruit, then allowing the fruit to be cold stored in air during the shipping period. When compared to CA the use of CO_2 shock treatment would be more cost effective but application would cause problems. At CO_2 concentrations between 20% and 50% and time periods for CO_2 treatment ranging

between 24 - 96 hours, our 'Fuerte' and 'Hass' avocados displayed no benefit in fruit quality from these treatments. The fruit may have been firmer than the control fruit, but this was far outweighed by the poor internal quality. This was possibly due to concentrations of CO_2 which were too severe and lengths of exposure to the elevated CO_2 levels which were too long, causing the fruit to be damaged very early in the experiment.

The dominance of CA storage during the export of South African grown avocados will soon be challenged by 1-methylcyclopropene (1-MCP). This ethylene antagonist acts by competitively inhibiting ethylene action, and due to its greater affinity for the ethylene receptor site than ethylene itself, it is effective at very low dosages. In contrast to this the increased level of CO_2 during CA storage acts as a non-competitive inhibitor of ethylene action. Our research clarified the benefits which can be gained by 'Fuerte' and 'Hass' avocados with CA or 1-MCP storage whether alone or in combination. Both the cultivars benefited from the treatments by improved firmness and shelf life while the 'Fuerte' avocados also had improved internal quality. The internal quality of the 'Hass' avocados was the best when the fruit were cold stored in air. From the results that we obtained there was not much to choose between treatment with either CA or 1-MCP. However, treatment of the fruit with 1-MCP is far more superior than CA storage in terms of ease of application and it is expected to be less expensive. The negative side to 1-MCP storage would be if the treatment were too effective and ripening is retarded to the extent that decay sets in before the fruit starts to ripen (White et al., 2001). However, 1-MCP is yet to be registered for commercial use and much still needs to be learned about the ability of 1-MCP during storage of avocados so as to utilise the product as effectively as possible.

While CA conditions during storage of avocados have shown promise for many years the control of relative humidity (RH) during storage has largely been neglected. This is partly due to the difficulties of measuring and controlling RH. Previous research has had positive results in firmness and colour retention, decreased disorder damage and shelf life extension. From our results, however, 'Fuerte' and 'Hass' avocados were not able to retain the quality of the fruit regardless of humidity and this outweighed any extensions in shelf life the fruit may have had.

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